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TABLE OF CONTENTS

WRIGHT, SEWALL, On the genetics of silvering in the guinea pig with special reference to interaction and linkage 387

PETERSON, P. A., Linkage of fruit shape and color genes in Capsicum . . 407

GRELL, R. F., Non random assortment of non-homologous chromosomes in *Drosophila melanogaster* 421

SERMONTI, G. and G. MORPURGO, Action of manganous chloride on induced somatic segregation in *Penicillium chrysogenum* diploids . 437

BANIČ, S., Transduction to penicillin and chloramphenicol resistance in *Salmonella typhimurium* 449

OZEKI, H., Chromosome fragments participating in transduction in *Salmonella typhimurium* 457

GARDNER, E. J., Genetic mechanism of maternal effect for tumorous head in *Drosophila melanogaster* 471

OGUR, MAURICE, RALPH ST. JOHN, SYLVIA OGUR and ABRAHAM M. MARK, The direct estimation of mutation rate from mutant frequency under special conditions 483

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ON THE GENETICS OF SILVERING IN THE GUINEA PIG WITH ESPECIAL REFERENCE TO INTERACTION AND LINKAGE[1,2]

SEWALL WRIGHT

Department of Genetics, University of Wisconsin, Madison, Wisconsin

Received July 7, 1958

THE genotypes of the major color varieties of a guinea pig colony, that had been almost self-contained since 1916, have been discussed in a number of papers (most recently, WRIGHT 1949a). The present paper will be concerned with a rather troublesome pair of alleles, *Sisi*, in which the heterozygote overlaps both homozygotes, its effects in interaction with other genes, and possible linkage relations. The most striking interaction effect, one with a hitherto unrecognized gene, will be reserved for a later paper.

The major color factors

The color factors that have been recognized in this stock may be characterized briefly as follows in terms of the effect on self-colored intense black (*E aa B C P S Gr Si*), self-colored intense brown (*E aa bb C P S Gr Si*), or on self-colored intense yellow or "red," (*ee C F S Gr Si*).

E,e^p,*e* e^pe^p: tortoise-shell (black and yellow), recessive to *E* (self black) and occasionally overlapping; *ee* self yellow, recessive to *E*; e^pe intermediate tortoise-shell.

A,*a* *A*-: agouti; black hairs with subterminal yellow bands on back; belly yellow, usually with some basal black; *A* dominant, no effect at birth in self-yellows (*ee*) or in yellow of tortoise-shell.

B,*b* *bb*: brown in place of black in fur, skin and eyes, *b* recessive. Accumulation of intensity factors (*C P F* and modifiers) beyond a certain point, results in a peculiar form of dilution known as dinginess.

C,c^k,c^d,c^r,c^a c^kc^k: very slight dilution of black (or brown), marked dilution of yellow.

c^dc^d: moderate dilution of black (or brown), yellow much as with c^kc^k.

c^rc^r: between c^kc^k and c^dc^d in dilution of black (or brown) in fur and skin, but eye color reduced more than by c^dc^d. White in place of yellow.

c^ac^a: albinism. White coat but developing dark pigmentation of feet, ears and nose; pink eyes.

C is completely dominant, while heterozygotes between lower *c* alleles are intermediate.

[1] Paper No. 728 from the Department of Genetics, University of Wisconsin.

[2] This investigation was aided by a grant from the WALLACE C. and CLARA A. ABBOTT Memorial Fund of the University of Chicago where the experimental work was conducted.

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P,p — *pp*: pink-eyed. Strong dilution of black (or brown) but no effect on yellow, *p* recessive in most combinations.

F,f — *ff*: fading yellow. Dilution of yellow much as in $c^k c^k$ at birth but fading later; no effect on black (or brown with a minor qualification) in the presence of *P*. In *E pp ff* there is replacement of sepia or brown by pale brownish cream, if *C* is present, otherwise by white. Gene *f* is not completely recessive.

S,s — *ss*: spotted. Colored areas on white ground, *s* incompletely recessive.

Gr, gr — *grgr*: grizzled. No effect at birth; progressive whitening of dorsal hairs later, *gr* incompletely recessive. Gene *gr* was not present in colony during period of these studies.

Si, si — *sisi*: silvered. Stationary sprinkling and blotching with white; slight dilution in unsilvered areas, *si* incompletely recessive. This pair is the main subject of the present paper.

Other loci, involved in linkage tests: It will be convenient to describe briefly here the effects of four loci, not concerned with color (except that *St* tends to produce a white spot on the forehead, which *R*, in conjunction with *m*, tends to inhibit (Bock 1950). These loci have been used in linkage tests with *Si,si*.

R,r — *R*-: Rough fur. Reversal of hair direction at least on toes, *R* fully dominant.

M,m — *M*: (more fully *M*(*R*), modifier of effect of *R*). *R-MM* shows low grade roughness (Rough E); *R-Mm* an intermediate grade (Rough C or D); and *R-mm* high grade roughness (Rough A or B). All are usually distinguishable in progenies.

St,st — *St*-: "Star." Strong forehead rosette. There is reciprocal partial inhibition by *R* and *St*. *St* is dominant (except in *R-mm St st*).

Px,px — *Px*: "Pollex." In *Pxpx* there is a tendency toward atavistic pentadactyly, of which presence of the thumb is most characteristic. This is independent of the common multifactorial atavistic little toe. *PxPx* is a lethal monster with short abnormal legs, hind legs rotated 180°, and up to 44 digits. There is also microphthalmia, and usually harelip and protruding brain.

Grades of intensity: Intensity of coat color has been recorded in the routine records made at birth by comparison with standard squares of dorsal skin (about $3' \times 3'$). These were chosen so that each grade is barely distinguishable from the preceding. Yellows have been graded from 1 for a pale "cream," barely distinguishable from white, to 13 for intense "red." The intensity modifiers necessary for grades 12 and 13 were absent from the colony during the period of the present studies. Sepias were graded by a second series of fur samples from 1, very pale sepia, barely distinguishable from white, to 21 for intense black. Browns differed so little in quality of coat color that they could be and were graded by the same series as the sepias.

Earlier conclusions on the genetics of silvering: Most of the inbred strains with which the author has worked (Wright 1922) have shown no trace of silvering.

One, however, (No. 34) consisted wholly of strongly silvered and brindled blacks (*$e^{v}e^{v}$ aa BB CC PP FF SS GrGr sisi*). Crosses of this strain with nonsilvered ones produced young that were usually slightly silvered on the belly. The proportions of wholly unsilvered and of strongly silvered young increased in F_2, but the upper 25 percent was much lower in average amount of silvering than strain 34. The results were interpreted as indicating multifactorial heredity (WRIGHT 1917).

IBSEN (1932), in a list of color factors of the guinea pig, attributed what seems to have been the same sort of silvering to an incompletely dominant gene, *Ro* (roan), but presented no data.

PICTET and FERRERO (1940) presented data on what also seems from their description and photographs to have been the same condition. They concluded that silvering is a heterogeneous category involving two independent pairs of alleles: dominant ventral whitening (*V*) and recessive dorsal silvering (*d*). They advanced the supplementary hypothesis that the type combination *vvD-* is lethal if both parents are *VvDd*, but not otherwise, to account for the absence of nonsilvered offspring from matings of such parents. Analysis of their data (WRIGHT 1947) indicated that these hypotheses were not acceptable and that the data were compatible with the hypothesis that there is a single basic gene for silvering, complicated by one or more modifiers that increase the degree of silvering and in so doing extend it from belly to back. Grades of silvering were not, however, given in PICTET and FERRERO's paper in sufficient detail for demonstration of the mode of inheritance.

Evidence for the hypothesis stated above was given by WRIGHT (1947) from the results of backcrosses, generation after generation, of nonsilvered or very slightly silvered segregants (*Sisi*) to a pure silvered strain (*sisi*). The gene *Si* maintained itself in spite of the continued attempt at dilution while strongly silvered segregants, mated *inter se* produced only silvers of highly varying grades.

IBSEN and GOERTZEN (1951) presented data that supported essentially the same hypothesis except that in their experiments some silvering (or "roan") was usually present in heterozygotes so that they preferred to treat the gene as dominant (*Ro*), while in my experiments reported in 1947, silvering was either absent or so doubtful in most heterozygotes that silvering was most conveniently treated as recessive (*sisi*). It was recessive in these experiments to about the same degree as spotting (*ss*) or grizzling (*grgr*) in both of which the heterozygotes may show traces and occasionally considerable of the "recessive" character and thus may overlap both homozygotes.

Grades of silvering: The following grades of silvering have been applied separately to back and belly, with the following definitions (1947). The total grade that will be used in this paper is the sum. A grouping was used in 1947.

grade 0 No silvering.
grade 1 Trace of silvering (irregular dilution or few white hairs).
grade 2 Slight but unmistakable silvering in a small area only.
grade 3 Silvering extensive but over less than half the area.

grade 4 Strong silvering or blotching with white over more than half of the area, but not approaching self white.

grade 5 Pure white predominates over the whole area.

grade 6 (new) Pure white (ventral). Pure white except on head (dorsal).

Evidence for single essential gene for silvering: The upper six rows of Table 1 largely repeat some of the data in the 1947 paper on the results of mating a silver strain I-13 with homozygous nonsilvers, with F_1 and successive low grade backcross segregants, and matings of the F_2 type. The table is, however, here restricted to young in which the ground color was black or black-red tortoise-shell, because of the greater uncertainty of grading on other backgrounds. This has led to removal of only a few individuals from the earlier table. Several records from later litters are added.

It may be noted that 63 percent of F_1 did not show even a trace of silvering. Only one is recorded as showing more than grade 2.

There are no significant differences among the successive backcrosses ($\chi^2 = 13.4$, ten degrees of freedom) but there is a bimodality in the last group (third to fifth backcross), absent in the first and second, that is suggestive. These results were interpreted as indicating that there is a single major gene, *Si*, that maintains its integrity through continued attempts at dilution. About 56 percent of the lower half of the total backcross population (roughly those that are *Sisi*) are still nonsilvers, and 95 percent of the upper half (largely *sisi*) are more silvered than all but one of the F_1 individuals, i.e., are of grade 4 or more.

TABLE 1

Grades of silvering in F_1 from matings between a silvered strain, I-13, and miscellaneous homozygous nonsilvered; in successive backcrosses (BC) of F_1 or low grade BC segregants to I-13; in F_2 or (BC)² from matings between low grade BC segregants; from extracted silvers mated inter se *or with I-13 (Ro early), and inbred derivatives (Ro late) from one mating of preceding; from descendants of outcrosses of Ro to other silvers (RoLF, RoXR). The average grades of the lower and higher parents, weighted by numbers of offspring, are given*

	Number matings	Lower parent			Higher parent			Grade of silvering							
		Geno.	Source	Av.	Geno.	Source	Av.	0	1–	3–	5–	7–	9–	11–	Total
F_1	7	*SiSi*	(misc)	0	*sisi*	(I-13)	7.3	43	24	1	..	..	..	..	68
1BC	10	*Sisi*	(F_1)	0.2	*sisi*	(I-13)	6.4	36	25	17	18	7	6	..	109
2BC	10	*Sisi*	(1BC)	1.0	*sisi*	(I-13)	6.8	14	14	13	12	7	1	..	61
3–5 BC	5	*Sisi*	(2–4BC)	0.9	*sisi*	(I-13)	6.4	12	15	4	7	8	6	..	52
F_2	4	*Sisi*	(F_1)	0.0	*Sisi*	(F_1)	0.4	13	6	2	..	1	..	..	22
(BC)²	8	*Sisi*	(BC)	0.7	*Sisi*	(BC)	1.0	25	47	9	10	4	..	..	95
Ro(early)	10	*sisi*	(BC,Ro)	6.1	*sisi*	(BC,Ro)	7.5	..	5	9	32	31	11	..	88
Ro(late)	9	*sisi*	(Ro)	7.8	*sisi*	(Ro)	8.9	..	..	..	3	74	38	..	115
RoLF	16	*sisi*	(LF,Ro)	7.0	*sisi*	Ro	8.7	..	2	8	31	71	44	(10)*	156
RoXR	8	*sisi*	(Ro)	8.2	*sisi*	(XR,Ro)	10.1	..	..	..	1	32	40	17	90

* RoLF produced ten white or near-white offspring that differed discontinuously and are not included in the total.

Those of the upper half are not, however, as strongly silvered on the average as the silvered strain Ro, derived from them and the parental silver strain I-13. It appears that there is multifactorial heredity at least in the sense that there are independent genes that modify somewhat the effects of segregation of the postulated major pair of alleles.

This impression is strengthened by comparison of these backcross arrays with the data of the F_2 type (matings between F_1's or between low grade segregants from the backcrosses $(BC)^2$). The upper 25 percent from the latter (roughly the *sisi* segregants) are much lower in average grade than the supposed *sisi* segregants from the backcrosses and of course still lower than the silver strains.

The distributions of the F_2 type are indeed so compact as to raise the question whether there may not, after all, be two loci with approximately equal effects. The persistence of a nearly or quite unsilvered class through the backcrosses could be due to selection of such low grade segregants that *both* type alleles were nearly always retained. On this view the original matings were of the type Si_1Si_1 $Si_2Si_2 \times si_1si_1$ si_2si_2 producing Si_1si_1 Si_2si_2 in F_1 and the successive backcrosses were largely or wholly Si_1si_1 $Si_2si_2 \times si_1si_1$ si_2si_2 by selection of the former. Such a hypothesis would obviously account well for the compact distribution of the F_2 type.

This compactness can also be accounted for, however, by segregation of a mere modifier of silvering (in a 1:2:1 ratio in F_2 as opposed to 0:2:2 in the backcross) in addition to that of a single essential gene for silvering. The relatively wide spread of the backcross arrays, the suggestion of bimodality in the later backcrosses point toward the latter hypothesis.

The modifiability of silvering is clearly shown by the significant difference between the distribution of the earlier ten matings of strain Ro, derived from high grade segregants and that of the later nine matings, tracing to a single mating of the earlier group.

Outcrosses (RoLF) were made to silvered reds of a strain (LF) in which silver of different origin was occurring. The character of the silvering seemed so different, consisting sometimes apparently of mere irregular dilution of red, that there was considerable doubt as to whether it was the same condition. The black-red crossbreds were, however, typical silvers. The distribution of silvering in these matings and inbred descendants (excluding a few reds for which the grades are less reliable) did not differ much from that in pure Ro, except for the production (by four matings) of five dark-eyed self-whites and five dark-eyed whites with strongly silvered sepia or yellow spots on one or both cheeks. The latter differs discontinuously from the strongest silvers in RoLF or Ro. The dark-eyed whites could not be accounted for as extreme spotted (*ss*) or as dark-eyed whites of genotype eec^rc^r (of which eight were produced by another of the matings). The ratio of ten white or near-white to 26 silvered black-reds and reds from these four matings suggests segregation of a recessive modifier that completely or almost completely eliminates all color from the coat of an animal of genotype *sisi*. Three of

this type had appeared in LF and many in other experiments which will be discussed in a later paper.

A mating, XR127, between rather high grade silvers (the sire from an outcross of Ro, the dam from F_2 of an outcross of RoLF) produced 19 silvers over the usual range (2 to 9) and one much more extreme (called grade 11) with pure white belly and dorsal silvering largely but not wholly restricted to the head. It was not, however, as extreme as the near whites from RoLF. This animal, a male, was mated with a female from Ro to start a group of eight matings (RoXR) with young of decidedly higher average grade than in any other silvered strain. In two of the matings both parents were of grade 11 and the average of their 12 offspring was 10.8, including three so nearly white that they were called grade 12. While these seem to bridge the gap between ordinary silvers and the whites and near whites of RoLF, they cannot be considered the same as the latter. As will be brought out elsewhere, males of the latter near-white or white category are sterile, and the near white males of RoXR are fertile.

On treating Ro, RoLF (near whites and whites excluded), and RoXR as a single strain, the regression of 436 offspring on midparent is .40 (.35 on sire, .43 on dam), indicating that about 40 percent of the variability is genetic and 60 percent nongenetic (including errors of grading) on the simplest hypothesis (additive modifiers with intermediate heterozygotes).

Further evidence has been given by matings of silvers from Ro and RoLF with animals with no silvering or merely a trace from a strain (A), made for the purpose of testing linkage of *Si,si* with certain loci. Strain A consisted of agoutis that were self-colored except for occasional silvering. The young in F_1 (Table 2) were agoutis and in the backcrosses, agoutis, agouti-reds, blacks, and black-reds. Four of the F_1 matings are interpreted as *SiSi* × *sisi*, eight as *Sisi* × *sisi*, and one (in which the A parent was of grade 2, higher than in any of the other cases) as *sisi* × *sisi*.

The results in F_1 and in the first backcross are rather similar to those of the previous set, except that the proportion, with no silvering at all, is less. Assignment of grade 1 instead of 0 is often, however, doubtful and especially in agoutis which have a yellow belly. The second backcross is more strongly bimodal than

TABLE 2

Grades of silvering in F_1 of cross between silvers of strain Ro or RoLF and nonsilvers or low grade silvers from strain A; two successive backcrosses of F_1 or low grade 1BC to Ro or RoLF

	Number matings	Lower parent			Higher parent			Grade of silvering							
		Geno.	Source	Av.	Geno.	Source	Ave.	0	1–	3–	5–	7–	9–	11–	Total
F_1	4	*SiSi*	(A)	0.0	*sisi*	(Ro,RoLF)	7.4	18	17	5	..	..	..	..	40
F_1	8	*Sisi*	(A)	0.2	*sisi*	(Ro,RoLF)	8.5	8	28	21	15	4	3	..	79
F_1	1	*sisi*	(A)	2.0	*sisi*	(Ro,RoLF)	6.0	..	4	..	4	..	..	..	8
1BC	13	*Sisi*	(F_1)	0.7	*sisi*	(Ro,RoLF)	7.8	14	49	33	37	35	9	(1)*	177
2BC	3	*Sisi*	(1BC)	2.0	*sisi*	(Ro,RoLF)	8.7	2	26	4	6	20	4	..	62

* A black-eyed white in 1BC, both parents of which traced to RoLF, is not included in the total.

even the third to fifth backcrosses in the previous set. This may be due to the fact that the low grade parent in each of the three matings had more than a trace of silvering (grade 2) though all proved to be *Sisi*. Apparently both parents in these matings had the same major modifiers favoring strong silvering, with the consequence that segregation was more nearly unifactorial than in the first backcross or in the earlier experiment. The fact that the mean of the lower half of this distribution was as low as 1.7, little more than a trace of silvering, confirms the view that there is a single basic factor for silvering. It may be added that matings have been made between silvers of as diverse origins as could be found in the colony. The results in all cases have indicated a single basic gene for silvering in this colony.

Interaction effects: As already noted, the effects of *sisi* are most conspicuous, and the grades accordingly most reliable, on a black ground. The trace of silvering of grade 1 is usually confined to the posterior belly and up to grade 4 silvering is in general confined to the belly except for a slight extension up the sides. There is sometimes great irregularity, and thus there may be black with only a trace of silver, if any, in an area on one side of the midline of the belly, white with a mere sprinkling of black hairs on the other. In the higher grades, the back may be black rather uniformly sprinkled with white, but the head and feet wholly black. More often there is irregularity: perhaps a white streak in the midline immediately surrounded by silvered black, or perhaps a compact spot of very strong silver on one shoulder or side, the rest, areas of self black and black with light silvering. Conversely the entire back may be strongly silvered except for a compact spot of black or lightly silvered black. Only in the highest grades, in which the belly and back are almost pure white does silvering invade the feet and head spots. Grades of intensity of color have been based on areas free of silvering.

Table 3 shows in condensed form the grades of silvering, their mean and the mean grades of intensity in unsilvered areas from genotypes that segregated out in all matings of type *sisi* × *sisi*, following outcrosses of strain Ro and RoLF, except that the yellows of genotype *C ff* are from matings of type *sisi* × *sisi* in strains of LF or RoLF and the agoutis and blacks in the last two rows are from the single mating XR127, on which RoXR was based.

The blacks show a rather high average grade of silvering (6.3) though not as high as in Ro (7.1), RoLF (7.3) or RoXR (8.9), undoubtedly because the favorable modifiers in these selected strains have been partly dissipated in the outcrosses. There is a slightly subnormal intensity (20.8, where 21.0 is standard intense black).

The browns show much silvering, but of lower average grade (4.9) than the blacks. The browns were difficult to grade satisfactorily because all of them were "dingy," a condition which somewhat simulates silvering (WRIGHT 1947). In dingy browns there is a sprinkling throughout the coat of hairs that are very pale brown but not white, except that there is intense brown for about a millimeter at the tip. The condition is due to factors *C*, *F*, and *P* (and modifiers) that intensify brown in most combinations but produce this peculiar form of dilution in higher

TABLE 3

Distribution of grades and mean grade of silvering and mean intensities in various colors and genotypes from matings of sisi × sisi, *except that eight* eeCff sisi *from* Sisi × sisi *are added to those from* sisi × sisi *for mean intensity. The agoutis and blacks in the last two rows are from a single mating (XR127). The 1949 norms (unsilvered) are given in the last column. In all genotypes with* E, *this may be replaced by* e^p

Color	Genotype	Grade of silvering 0	1–	3–	5–	7–	9–	11–	silvering no.	silvering m.	intensity no.	intensity m.	1949 norm
Black	*E B C P*	..	3	9	16	36	6	..	70	6.3	60	20.8	21.0
Brown	*E bb CC PP*	2	8	13	30	15	1	..	69	4.9	40	12.7	15.3
Pale Sepia	*E B C pp*	2	3	3	14	14	6	..	42	5.9	39	9.7	9.7
Dark Sepia	*E* Bc^dc^dP	..	..	1	11	11	2	..	25	6.6	24	17.5	18.1
Light Sepia	*E* Bc^dc^aP	..	4	2	2	..	..	..	8	2.9	8	13.7	14.5
V.P. Sepia	*E B* c^dc^d *pp FF*	..	1	..	5	..	..	..	6	4.8	6	6.2	4.7
V.P. Sepia	*E B* c^dc^a *pp FF*	4	..	..	..	..	..	..	4	0.0	4	2.5	2.7
Red	*ee-C-FF*	1	6	10	9	10	4	..	40	5.1	39	9.5	10.6
Yellow	*ee-C-ff*	..	..	1	1	2	1	..	5	6.6	13	6.1	6.8
Cream	*ee-*c^dc^a*-FF*	1	1	..	..	2	..	..	4	4.0	4	4.2	4.2
Agouti	*A E B C P*	..	..	4	1	3	2	..	10	6.3	..	...	...
Black	*aa E B C P*	..	1	1	3	3	1	1	10	6.1	..	...	...

combinations and most in *CC FF PP* which all of the browns in Table 3 were. The low intensity of these silvered and dingy browns (12.7) in comparison with 1949 norm (15.3) is undoubtedly due largely to the dinginess. All animals with more than a trace of dinginess were excluded in the data for the 1949 average.

There was no significant correlation between the grades of silvering and of dinginess ($-.05 \pm .13$). It is, however, probable that the grade of silvering tended to be reduced slightly by confusion with dinginess. There is no satisfactory evidence for any real interaction.

The pink-eyed pale sepias are not substantially less silvered than the blacks (5.9 *vs.* 6.3) and have the same average intensity (9.7) as the 1949 norm for their genotype. The apparent absence of any dilution may, however, be due to an unduly low 1949 norm since modifiers that reduce the intensity of pale sepias were important in the early history of the stock, but not later. The average for nonsilvered pale sepias during the period of these studies was about 10.4. With respect to the grade of silvering, that for the belly was often doubtful because of the paleness of the ground color. The conclusion that seems warranted is that the effects of *pp* and *sisi* are approximately additive.

The silvered dark-eyed sepias with c^dc^d also show substantially the same grade of silvering as the blacks (6.6 *vs.* 6.3). They are distinctly below the 1949 norm for their genotype in intensity (17.5 *vs.* 18.1). The apparently greater dilution of color with c^dc^d than with *C*, may merely reflect a saturation effect in intense black. The simplest interpretation is again that there is no interaction, but greater dilution of c^dc^d than *C* by *sisi* is at least suggested.

The few light sepias (c^dc^a) from these matings show a decidedly low grade of silvering but only slightly more dilution than the 1949 norm. This low grade of silvering is borne out by observations of some 200 dark-eyed and red-eyed light sepias (c^dc^a and c^rc^a) that have been produced from matings from which silvered segregants were expected. Not one of them was recorded as having more than a trace of silvering on the belly. The four cases in Table 3 with silvering of grades 3 to 6 seem in fact to have been the only definitely silvered light sepias of genotypes c^dc^a or c^rc^a to have been recorded in any mating. Among three other cases of c^dc^a from *sisi* × *sisi*, not in the series tabulated, not one showed a trace of even ventral silvering.

The way in which silvering seems to go out of sight in most matings when combined with c^dc^a in a sepia may be illustrated by a pair of matings in which one of the most strongly silvered c^dc^d sepias (grade 9) in Table 3, a female, was mated with an intense yellow (*ee C* c^a *SiSi*). A son, sepia 13-yellow 4, c^dc^a *Sisi* was mated back to her. None of the 13 offspring showed more silvering than grade 2. Four were dark sepias (one grade 16, three grade 17) identified as c^dc^d by yellow areas of grade 7, and nine were light sepias (three grade 12, six grade 13) identified as c^dc^a by light yellow spotting of grade 4 or less. The absence of overt silvering in c^dc^d of higher grade than 2 (and only one of this grade) is surprising but may have been a chance result. The absence in the c^dc^a's (again only one as high as grade 2) is highly unlikely as a chance event. The lighter ones (grade 12) may have been *sisi* and the others (grade 13) *Sisi*, but exact assignment gives more weight to the grades in individual cases than is warranted.

The absence of overt silvering in most c^dc^a and c^rc^a sepias cannot be merely an illusion due to their light color since dorsal silvering is conspicuous in most pink-eyed sepias of genotype *E C pp FF sisi* which are considerably lighter. Moreover, the color of c^dc^a and c^rc^a sepias differs little from that of browns in which dorsal silvering is usually conspicuous in spite of the complication from dinginess. It seems to be certain, therefore, that there is an interaction between *sisi* and c^dc^a (and probably also c^rc^a) by which silvering is much reduced. Its presence in four young from the Ro outcrosses of Table 4 but absence elsewhere in the colony may be due to more favorable modifiers in the former derived from the selected silvers of Ro.

There is also evidence from analysis of reflectionmeter readings of intensity, not presented in this paper, that the intensity of c^dc^a and c^rc^a sepias is usually reduced more than indicated in Table 3 for the former. This effect is especially apparent in combination with another modifier, *dm*.

There were six pale-sepia yellows (c^dc^d *pp*) from these matings of *sisi* × *sisi*. Five showed moderately strong silvering while one showed only a trace. Four pale sepia-yellows of genotype c^dc^a *pp* from these matings showed no recognizable silvering. There was no difference in intensity from the norm that could be relied upon but again the norm was undoubtedly based in part on animals with modifiers favoring dilution. As far as these data go, they confirm the absence of inter-

action effects of *pp* and c^dc^d on *sisi* and the tendency toward suppression of silvering when c^dc^a is combined with *sisi*.

These matings produced 40 silvered intense yellows (*ee C-FF*). These include some with silvering as strong as observed in blacks but the average is considerably lower. The silvered intense yellows are also considerably lighter in color than the norm. Low grade silvering is difficult to grade in these since silvering is manifested more by a rather uniform lightening of the color of the belly than by intermingling of white and colored hairs. There is sometimes, however, marked irregularity in intensity. As with all of the other light colors, a microscopic study of the distribution of pigment within and among hairs would be required to determine how much real difference there is from the effect of silvering in blacks. To a first approximation the effects are additive.

Matings of *ee CC ff sisi* interse produced four silvered fading yellows all of high grade in an early series of experiments (LF) and one of these segregated out from a mating of *ee CC Ff sisi* interse in RoLF. The average (6.6) is about the same as for silvered blacks in the Ro outcrosses. Five *ee CC ff sisi* segregants from matings of *Sisi* × *sisi* (and *ff* × *ff* or *Ff* × *ff*) were also well silvered. The average grade of silvering for all ten was 6.0. The average grade of intensity of these and three others from the first group, born dead and not graded in silvering, was 6.1 which is somewhat lighter than the norm (6.8). There is no evidence for anything other than additive effect of *ff* and *sisi*.

No yellows of genotype *ee* c^dc^d *FF sisi* were produced by the Ro outcrosses. A great many have been produced in other series in which dilution factor *dm* was often present. High grade silvering has often been present but as with the intense yellow offspring from silvered parents, they rather often show no evidence of silvering except a decided lightening of the color on the belly and some lightening of that on the back.

Four light yellows of genotype *ee* c^dc^a *FF sisi* were produced. While two showed no more than a trace of silvering, two were conspicuously silvered on the back as well as almost white on the belly. A mating (L257) between two light yellows of grade 4 (both *ee* c^dc^a *sisi*) and four matings derived from this in two generations of brother-sister mating produced 39 light yellow young of grades 3 and 4 (average 3.56) as well as albinos. Nine young of grades 5 and 6 (average 5.36) came from two of the matings that were $c^dc^a \times c^dc^a$, and are interpreted as c^dc^d. These averages are decidedly lighter than the norm. Several were described as having very light but not white bellies, and a few as being somewhat irregular in intensity on the back, but silvering with white was not recognized in any of them. There is also evidence that light yellows of genotypes c^kc^r, c^kc^a, and c^dc^r are closely similar. These results agree with those from the c^dc^a sepias that there is a nonadditive interaction between *sisi* and lower combinations in the c-series under which silvering with white is largely suppressed, but dilution, especially of the belly, is enhanced.

The combination of medium silvering (*sisi*) with low to medium spotting (*ss*) shows an approximately additive effect in blacks: spots of pure black and of

lightly silvered black on a white ground of the same extent, as if silvering were absent. Low grade silvering is often undetectible in spotted animals since pigment tends to be eliminated more on the belly in the latter than in other regions, except feet and nose. In high grade spotted animals, spots are likely to be restricted to the region about the eyes and ears. As these same regions are those least likely to be silvered, even rather high grade silvering may be undetected in high grade spotting.

On the other hand the fact that high grade silvering may be reponsible for large, pure white areas means that animals that would be only low to medium grade spotted otherwise, may appear to be high grade spotted because of this effect of *sisi*. In silvered medium spotted animals, there are apt to be one or more spots of smaller size than are found on unsilvered spotted. In this sense, silvering may act as a member of the group of modifiers of spotting.

There are indications, moreover, that there is some genuine nonadditive interaction such that silvering tends to invade areas (such as the eye-ear region) in high grade spotting to a greater extent than in the absence of such spotting. There is also evidence of interaction in tricolors. An unspotted tortoise-shell (c^pc^p *SS*) is usually a black with scattered small clumps of yellow, or isolated yellow hairs. The interaction, if any, with silvering is merely what one would expect from the appearance of silvering in self-blacks and in self-reds. Thus sprinkling with white is more conspicuous in the black areas while dilution is more conspicuous in the yellow areas of tortoise-shells. In tricolors, (e^pe^p *ss*), the tortoise-shell pattern is profoundly modified (WRIGHT 1917; ILJIN 1928; CHASE 1939). The amount of yellow is greatly increased and there is a tendency toward separate black and yellow spots on the white ground or else of black spots on a ground that is in part yellow and in part white. A streak between two black areas is often white at one end, separated sharply from yellow at the other.

The boundaries of spots of strong and weak silvering in tricolors tend to be the same as those between black and yellow. Examination of the records of the patterns of tricolors in Ro and its outcrosses revealed only four cases in which silvering was stronger on the black side of such a boundary but 14 cases in which silvering was stronger on the yellow side (contrary to the usual situation in tortoise-shells). The latter cases suggest that the effect of *ss* that favors yellow also favors silvering.

Presumably the same sort of interaction occurs in black piebald (*E-ss*) and tends to increase both the strength of silvering and the amount of white. It is probably no accident that the most extreme spotted inbred strain in the colony (#13) with 97.8 percent as the median percentage of white, frequent silvering of the head spots, and 24 percent self-white (WRIGHT and CHASE 1936) turned out to be *ss sisi* on outcross to self-colored animals. It was in fact the source of the silvering in strains I-13 and Ro.

It should be emphasized that these interaction effects are second order ones. To a first order, silvering and spotting are additive.

The last two rows of Table 3 compare the amount of silvering in agoutis and

blacks (some of them tortoise-shells), from a single mating XR127. There is evidently no recognizable difference.

Tests for linkage of Sisi *with other loci:* Tests for possible linkage have been made previously in every possible pair among the following loci: *S, E, A, B, C, P, F, R, M, St, Px,* and *Sex* (WRIGHT 1941 and 1949b). A significant deviation from 50 percent recombination has been found in only one case *viz. R* and *Px* with 43.8 percent recombination among 1015 young. None of these genes showed any indication of partial sex linkage of any sort. We consider here tests for linkage of *Si*.

The prevailing genes in strain Ro and RoLF were e^e, *a, B, C, P, F, S, si, r,* and *px,* but *ee,* $c^r c^r$ and *ss* segregated out from some matings. *R* was present in some matings and *M* and *m,* which are unrecognizable except in the presence of *R,* were both present. Strain A was characterized by *E, A, B, C, P, F, S, R,* and *St.* Both *M* and *m* were present. Many animals were *Pxpx.* Silvering as already stated was not uncommon. Backcross progenies from *Ro* × *A* gave coupling data for *Si* in relation to *E, A, R, St,* and *Px* but not always from the same mating.

A considerable number of matings were made between silvers from Ro and nonsilvers that were *bb,* $c^d c^d$, *ff, pp,* or *ss,* sometimes in combination. The F_2 repulsion data from these matings was much less satisfactory than the backcross data because of the greater amount of overlap characteristic of F_2, as already discussed. Double recessive strains *bb sisi* and *pp sisi* (used in the discussion of interaction) were, however, established in time to permit the obtaining of some backcross coupling data.

Search through the records for matings of backcross types yielded some repulsion data as well as a few supplementary coupling data.

The linkage tests for *M* and *Si* had to be based on a rather small number of matings from which certain classes of rough-furred young could be shown by pedigree analyses to give valid evidence.

In the progenies of the backcross type, the cleavage between those treated as *Sisi* and those treated as *sisi* was in general made between silver grades 3 and 4 in order to give reasonably good 1:1 ratio. There were four large progenies, however, (not involving the selected silvered strains) in which this cleavage was made between 2 and 3 or between 1 and 2 for the same reason. In the case of the F_2 repulsion data, cleavage had to be made between grades 1 and 2 in all cases to give reasonably good 3:1 ratios. The same cleavage was used throughout for any given mating.

The distribution of grades is given in condensed form in Tables 4 to 6. The estimates of the number of recombinants (parentheses) and nonrecombinants depend on the points of cleavage chosen for the separate matings as indicated above and thus cannot be obtained from the distributions as given.

The matings of *Sisi* male by *sisi* female have been examined for possible partial sex linkage of the expected type. Neither the coupling nor the repulsion data indicate linkage. Altogether, there are estimated to be 277 recombinants in 528 young or 52.5 percent (Dev = 1.1 SE). The reciprocal matings have also been

TABLE 4

Backcross tests for linkage of Si *with sex (heterogametic male or heterogametic female)* Px, R, St, E, A, B, P, *and* C. *The number of recombinants, as given by the chosen points of cleavage, are put in parenthesis under* Sisi *and* sisi

	Mating type ♂ ♀	No. matings		0	1–	3–	5–	7–	9–	11–	Total	*Sisi*	*sisi*	Percentage recomb.		Dev. / SE
♂C	*Sisi* × *sisi*	24	♂	21	49	27	25	29	11	2	164	74	(90)	51.4	52.5	1.1
			♀	17	52	27	35	29	5	2	167	(80)	87			
♂R	*siSi* × *sisi*	20	♂	29	26	14	19	13	6	1	108	(57)	51	54.3		
			♀	15	24	15	21	8	5	1	89	39	(50)			
♀R	*sisi* × *Sisi*	8	♂	11	7	6	6	5	1	..	36	18	(18)	52.2	54.2	1.5
			♀	11	5	2	6	9	..	..	33	(18)	15			
♀C	*sisi* × *siSi*	29	♂	32	42	24	24	4	5	2	133	(79)	54	54.8		
			♀	24	36	20	22	15	2	..	119	60	(59)			
♀C	*Px Si* × *px si*		*Pxpx*	2	13	4	12	4	1	..	36	16	(20)	55.3		
	px si *px si*	6	*pxpx*	7	11	5	9	8	..	..	40	(22)	18			
♂R	*Px si* × *px si*		*Pxpx*	3	2	1	2	3	1	..	12	(6)	6	39.3	51.2	0.3
	px Si *px si*	2	*pxpx*	5	6	..	2	2	1	..	16	11	(5)			
♀R	*px si* × *Px si*		*Pxpx*	1	1	4	..	..	..	..	6	(1)	5	52.9		
	px si *px Si*	2	*pxpx*	2	2	4	3	..	..	..	11	3	(8)			
♂C	*R Si* × *r si*		*Rr*	26	50	28	32	39	8	..	183	91	(92)	49.3		
	r si *r si*	22	*rr*	24	50	30	43	26	13	..	186	(90)	96			
♀C	*r si* × *R Si*		*Rr*	13	10	8	5	3	1	..	40	27	(13)	47.3	48.9	0.5
	r si *r si*	10	*rr*	12	13	9	9	6	2	..	51	(30)	21			
♂C	*St Si* × *st si*		*Stst*	7	18	21	12	11	2	..	71	36	(35)	44.7		
	st si *st si*	10	*stst*	8	15	15	11	14	7	..	70	(28)	42			
♀C	*st si* × *St Si*		*Stst*	13	17	12	14	3	3	..	62	36	(26)	48.3	46.4	1.2
	st si *st st*	13	*stst*	12	18	9	11	7	1	..	58	(32)	26			
♂C	*E Si* × *e^p si*		*Ee^p*	8	31	20	25	21	4	..	109	55	(54)	48.7		
	e^p si *e^p si*	14	*e^{p}e^p*	7	44	17	18	27	10	..	123	(59)	64			
♂R	*E si* × *e^p si*		*Ee^p*	..	2	..	3	1	..	..	6	(2)	4	57.1		
	e^p Si *e^p si*	1	*e^{p}e^p*	..	2	1	2	1	2	..	8	2	(6)			
♀C	*e^p si* × *E Si*		*Ee^p*	12	20	14	22	7	6	..	81	35	(46)	53.5	51.4	0.6
	e^p si *e^p si*	19	*e^{p}e^p*	18	23	15	20	9	4	..	89	(45)	44			
♀R	*e^p si* × *E si*		*Ee^p*	9	11	1	..	4	..	..	25	(15)	10	56.4		
	e^p si *e^p Si*	1	*e^{p}e^p*	2	8	2	1	1	..	..	14	7	(7)			
♂C	*A Si* × *a si*		*Aa*	17	49	18	24	25	9	..	142	77	(65)	43.1		
	a si *a si*	20	*aa*	9	39	31	36	33	7	..	155	(63)	92			
♂R	*A si* × *a si*		*Aa*	6	8	2	1	1	..	..	18	(10)	8	48.7	43.7	2.4
	a Si *a si*	1	*aa*	5	11	1	..	3	1	..	21	12	(9)			
♀C	*a si* × *A Si*		*Aa*	2	3	..	2	1	2	..	10	5	(5)	42.9		
	a si *a si*	6	*aa*	3	3	3	4	3	2	..	18	(7)	11			
♂C	*B Si* × *b si*		*Bb*	..	1	2	..	1	1	..	5	1	(4)	55.6		
	b si *b si*	2	*bb*	..	1	..	2	1	..	..	4	(1)	3			
♂R	*B si* × *b si*		*Bb*	..	..	..	1	..	..	..	1	(0)	1	50.0		
	b Si *b si*	1	*bb*	..	..	..	1	..	..	..	1	0	(1)			
♀C	*b si* × *B Si*		*Bb*	1	6	2	3	1	..	..	13	7	(6)	55.6	50.0	0.0
	b si *b si*	4	*bb*	3	1	..	1	..	..	..	5	(4)	1			
♀R	*b si* × *B si*		*Bb*	5	2	3	2	1	..	..	13	(7)	6	42.9		
	b si *b Si*	3	*bb*	4	2	..	2	..	..	..	8	6	(2)			
♂C	*P Si* × *p si*		*Pp*	3	5	5	10	8	3	..	34	11	(23)	60.8		
	p si *p si*	5	*pp*	6	12	8	12	1	1	..	40	(22)	18			
♂R	*P si* × *p si*		*Pp*	7	6	4	3	..	..	..	20	(8)	12	40.0	53.9	1.0
	p Si *p si*	1	*pp*	8	8	3	1	..	..	..	20	12	(8)			
♀C	*p si* × *P Si*		*Pp*	3	6	4	6	2	2	..	23	9	(14)	54.9		
	p si *p si*	5	*pp*	1	11	5	7	2	2	..	28	(14)	14			
♂C	*C Si* × *c^d si*		*Cc^d*	..	2	..	3	..	..	..	5	2	(3)	55.6		
	c^d si *c^d si*	2	*c^{d}c^d*	..	1	1	1	1	..	..	4	(2)	2			

examined on the improbable hypothesis that the female may be heterogametic. There is no indication of linkage (174 estimated recombinants in 321 or 54.2 percent (Dev = 1.5 SE).

In the case of *Si* and *Px*, there is no indication of linkage from either coupling or scanty repulsion data. Altogether 62 recombinants in 121 young are indicated or 51.2 percent (Dev = 0.3 SE).

Only coupling data were obtained for *Si* and *R*. There is no indication of linkage either in tests of F_1 males or F_1 females, or in both combined (225 recombinants in 460 young or 48.9 percent, Dev = 0.5 SE).

Similarly there is no indication of linkage of *Si* with *St* in backcross coupling tests of either F_1 males or F_1 females. The estimated proportion of recombinants altogether is 121 in 261 or 46.4 percent (Dev = 1.2 SE).

In the case of *Si* and *M*, four matings were found within the backcrosses to I-13, five matings were found in the backcrosses of A to *Ro* and one additional mating (XR161) was found in the records, which, by pedigree analysis, could give information (Table 5). No information is given by the smooth young. All of the matings were of the type *Sisi* × *sisi* but only two were *Mm* × *mm* and two *MM* × *Mm* permitting use of all of the rough-furred young. Among the others, two were *Mm* × *Mm*, three were *Mm* × *M-* and one was *Mm* × *m-*, where the dash represents uncertainty. In these last six cases, only high grade (*R mm*) and low grade (*R MM*) roughness gives information on linkage. The cleavage between those interpreted as *Sisi* and as *sisi* was taken at the same points as in the other tests (between grades 3 and 4 in three cases, between grades 2 and 3 in five cases, including one not otherwise used, and between grades 1 and 2 in two cases).

On this basis there are 20 recombinants in 92 usable offspring or 21.7 percent (Dev = 5.4 SE). This clearly demonstrates linkage if the assignment of genotypes is acceptable. No plausible shifts in the points of cleavage chosen for *Sisi* and *sisi* were found to have any important effect on the result. Another complication is the occasional overlap of *R Mm* and *R MM*. Thus the last mating in Table 5; *mm* × *Mm*, produced one offspring called Rough E which is usually *R-MM* but occasionally *R-Mm* with which it must here be counted. Examination of the pedigrees for possible errors of this sort, revealed none which would affect the result appreciably. The female of XR161 was a pale cream of grade 1 in which silvering cannot be recognized except by the extreme dilution. She could have been *Sisi* (*dmdm*) instead of *sisi* (*Dmdm*) from her parentage (*dm* being a modifier of intensity). In this case only the silvered young should be counted, removing two recombinants and eight nonrecombinants from those used. The ratio of silver to nonsilver (9:10) makes the interpretation in the table more probable.

In the case of *Si* and *E*, considerable coupling data and a few repulsion data were obtained for both sexes with no indication of linkage in any. The total estimated number of recombinants was 234 in 455 or 51.4 percent (Dev = 0.6 SE).

There is some indication of linkage of *Si* and *A*. Altogether there are estimated to be 159 recombinants in 364 or 43.7 percent (Dev = 2.4 SE). The probability of as great a derivation in this direction from accidents of sampling is about .008.

TABLE 5

Ten matings which give information on possible linkage of Si *and* M. *All young are included, but those which give no information on linkage are in parentheses. In formulae of parents, genes from sire in numerator, from dam in denominator. Uncertain allele represented by dash,* C *coupling,* R *repulsion. The totals indicate 21.7 percent recombination*

	♂ × ♀		*R-mm*	*RMm*	*RMM*	*Sm (rr)*	Total	Non Rec.	Rec.
Ro38	*si m* × *si M*	*Sisi*	..	..	3	(4)	7	3	..
(♂C)	*Si M* / *si* —	*sisi*	..	(2)	0	(5)	7	..	..
Ro51	*si m* × *si M*	*Sisi*	..	..	1	(3)	4	1	..
(♂C)	*Si M* / *si* —	*sisi*	..	..	1	(1)	2	..	1
SiAll	*si M* × *Si M*	*Si si*	..	0	2	(2)	4	2	..
(♀C)	*si M* / *si m*	*si si*	..	2	0	(0)	2	2	..
SiA51	*Si m* × *si M*	*Si si*	..	1	..	(1)	2	1	..
(♂R)	*si M* / *si M*	*si si*	..	2	..	(6)	8	..	2
Ro16	*si M* × *si M*	*Si si*	..	(1)	..	(4)	5	..	..
(♀R)	*si* — / *Si m*	*si si*	..	(2)	2	(3)	7	2	..
SiA47	*si M* × *si M*	*Si si*	7	(7)	?	(4)	20	7	2
(♂R)	*Si m* / *si m*	*si si*	1	(9)	7	(2)	19	7	1
SiA39	*si M* × *si M*	*Si si*	10	(6)	4	..	20	10	4
(♀R)	*si m* / *Si m*	*si si*	3	(8)	5	..	16	5	3
SiA43	*Si m* × *si m*	*Si si*	7	1	..	(18)	26	7	1
(♂R)	*si M* / *si m*	*si si*	..	11	..	(10)	21	11	..
Ro2	*Si m* × *si* —	*Si si*	1	(2)	..	(4)	7	1	..
(♂R)	*si M* / *si m*	*si si*	..	..	..	..	0	..	..
XR161	*si M* × *si m*	*Si si*	8	2	..	..	10	8	2
(♂R)	*Si m* / *si m*	*si si*	4	5	..	..	9	5	4
								72	20

The probability for a random pair of loci to show linkage with recombination as great as 43.7 percent is, however, also small, noting that the guinea pig has 33 pairs of chromosomes and assuming that recombination percentages are distributed uniformly between zero percent and 50 percent. This probability is .0019 ($= .030 \times .063$). The probability for such a pair of loci to be in a different chromosome and yet to show as little recombination as 43.7 percent is .0078 ($= .970 \times .008$) or four times as great. The *Si* and *A* loci cannot perhaps be considered as a random pair since they show next to the strongest indication of linkage among nine somewhat adequately tested cases discussed here. Nevertheless it is clear that it is at least as likely that this is a deviation due to accidents of sampling as that it is due to linkage. There are, however, other possibilities.

One such possibility is that there is linkage between *A* and a modifier of silvering that makes for a low grade in comparison with its allele from the high grade selected strain Ro. The difference between the mean grades of silver of *Aa*(4.0) and *aa*(4.2) in the first backcross generation in which one might expect linkage to be shown most clearly, is however, less than in the remainder of the coupling data (second backcross and miscellaneous matings) (*Aa* 3,5, *aa* 4.4).

The most probable explanation seems to be a slight systematic bias in grading silvered agoutis as compared with silvered blacks. Traces of silvering are more easily observed on the black belly of *aa* than on the yellow belly of *Aa*. Such a bias would be most serious in the low grades which are thus likely to be underestimated in agoutis. These grades were not represented to an important extent in the previously discussed comparison of *Aa* and *aa* from *sisi* × *sisi*.

The evidence for random assortment of A and M (50.4 percent recombination among 607 young, WRIGHT 1941) and that for strong linkage of *Si* to *M* (ca. 22 percent), presented here, also make it unlikely that *Si* and *A* are in the same chromosome.

Table 6 shows the results in F_2 from matings of *BB sisi* × *bb SiSi*. It is evident that recombination occurs. If cleavage between *Si*- and *sisi* is made between grades 1 and 2, the estimated proportion of double recessives, seven in 125, excluding seven near whites is approximately that expected under random assortment (expected 7.8). There is, however, so much overlap between *Sisi* and *sisi* in F_2 due to segregation of modifiers that little stress can be put on an F_2 ratio. A double recessive strain was established, and a few backcross data obtained which are of more value (Table 4). The coupling and repulsion data from F_1 males and females

TABLE 6

F_2 data on possible linkage of Si *with* B, P, F, S, *and* C. *Near-white (unclassifiable but with excess likelihood of being* sisi *in parentheses). Trace of silvering difficult to recognize in most cases. Any silvering probably unrecognizable in* ffpp. *Cleavage in all cases between grades 1 and 2*

Type of F_2 mating	Number matings		Grade of silvering 0	1–	3–	5–	7–	9–	Near W	Total	0–1	2–9
Repulsion	9	*B–*	46	25	6	7	4	..	(5)	88	63	25
		bb	24	10	1	1	1	..	(2)	37	30	7
Coupling	3	*P–*	8	9	3	1	1	1	...	23	16	7
		pp	4	3	..	1	..	..	...	8	7	1
Repulsion	3	*P–*	23	14	1	1	2	..	(1)	41	34	7
		pp	16	4	1	1	..	..	...	22	18	4
Repulsion	5	*F–P–*	23	17	2	2	2	..	(2)	46	37	9
		ffP–	12	3	..	..	..	..	(4)	15	13	2
		F–pp	8	3	..	1	..	..	...	12	9	3
		ffpp	6	..	..	..	..	..	...	6	6	0
Repulsion	8	*S–*	58	36	11	4	2	..	...	111	81	30
		ss	19	6	..	2	..	..	(9)	27	19	8
Repulsion	3	*C–*	4	10	5	1	2	1	...	23	14	9
		c^dc^d	2	6	..	1	..	..	...	9	8	1

all indicate that recombination occurred freely. The over-all number of recombinants is estimated at 25 in 50 or exactly 50.0 percent.

In the case of *Si* and *P*, F_2 data were obtained for both coupling and repulsion (Table 6). On making the cleavage between 1 and 2, the repulsion data yielded four in 63. There is clearly no indication of linkage. Both coupling and repulsion data were also obtained (Table 4) from backcrosses to the double recessive strain derived from F_2. Again there is no indication of linkage (89 estimated recombinants in 165 young or 53.9 percent) (Dev = 1.0 SE).

As noted earlier, spotting (*ss*) on the one hand interferes with the expression of *sisi* by eliminating color from the regions in which silvering tends to be strongest, and on the other, there are indications of an interaction effect by which *ss sisi* tends toward self-white or near-white somewhat more frequently than *ss Si-*. For both reasons simple linkage tests are certain to be unsatisfactory. However, matings were made between *SS sisi* from strain Ro and unsilvered spotted animals *ss SiSi* and carried to F_2. As far as they go, the results indicate that recombination occurred at random. The arbitrariness of the cleavage between *Ss* and *ss* (between 4 and 5 parts of white in 20), and between *Sisi* and *sisi* (between silver grades 1 and 2) and the production of nine black-eyed whites or near whites (*ss*), that could not be graded in silvering, make this ratio highly uncertain. The double recessive strain (inbred strain 13) was not on hand at the time linkage was being tested and no backcrosses were made. The fact that *SS sisi* was easily extracted from outcrosses of strain 13 (to produce strain I-13) indicates that linkage is not close.

An F_2 test for possible linkage of *Si* and *F* was attempted from matings of *FF sisi* × *ff SiSi*. Unfortunately both *ss* and *pp* segregated from F_2 to complicate classification. There were six near whites (of which four were *ss ff*) in which silvering is unrecognizable. Silvering would probably also be difficult or impossible to recognize in *E ff pp* which is very pale cream. These are tabulated separately. Among those with *P-* and not near white, only two double recessives, *ff sisi*, were recognized among 61 young, and these were borderline silvers (grade 2). The data are thus compatible with any degree of recombination. No double recessive strain was established at this time and no backcross data are on hand. The fact that there was no difficulty in producing double recessives at an earlier period (strain LF referred to in connection with possible interaction effects) shows that recombination can occur.

Tests for linkage of *Si* with *C* were delayed by failure to recognize that *sisi* is usually not recognizable with confidence in $c^d c^a$. Only a few F_2 young were obtained from the mating *CC sisi* × $c^d c^d$ *SiSi*. There was one clear case of a double recessive (silver grade 5) among 32 young and no border line cases. In a mating of backcross type (coupling) there were five recombinants among nine young (cleavage between grades 2 and 3). There is an abundance of data indicating easy transfer of *si* from one *c* allele to another but uncertainties of classification make these useless for calculation of recombination percentage.

The best evidence at present that *Si* is not linked with *S*, *F*, or *C* comes from

the evidence for rather strong linkage with *M* and the earlier evidence that *M* shows random assortment with these (52.9 percent with *S* in 363 backcross young, 56.7 percent with *F* in 157 backcross young, and 48.7 percent with *C* in 275 backcross young).

SUMMARY

The character "silver" in the guinea pig ranges from a few white hairs on the belly almost to self-white. Whatever the grade, it is present at birth and not progressive. Data have been given in a previous paper indicating that self or low grade silvering maintains itself through repeated backcrosses to a high grade silver strain as if dependent on a single incompletely dominant gene. This result is confirmed here by data from another cross, carried to the second backcross. In the latter the distribution was definitely bimodal. F_2 from self by high grade silver gave no clear indication of unifactorial segregation because of segregating modifiers.

The existence of genetic modifiers is shown by the rapid advance under selection. The regression of offspring on midparent was .40 in a group of related strains that extended over the entire range.

The silvering character is often highly irregular especially in intermediate grades in which corresponding areas on the right and left sides may differ greatly. It is convenient to distinguish this "blotching" effect from the silvering in the narrow sense. There is also some dilution of the intensity of color in nonsilvered (as well as silvered) areas. The silvering is most conspicuous on a black ground, while the dilution is barely perceptible in blacks. The effect of the gene seems to be essentially the same in browns (*bb*), pale sepias (*pp*), and dark sepias (c^dc^d) although silvering becomes more difficult to recognize on the paler backgrounds, and dilution becomes more conspicuous (perhaps merely because of saturation in blacks). In light sepias (c^dc^a, c^rc^a), however, silvering tends to disappear and uniform dilution becomes the most characteristic effect. Silvering may be extreme but is usually less expressed in intense yellow (*ee*) or dilute yellows (*ee* c^dc^d and *ee ff*) than in black while dilution is more conspicuous.

In still paler yellows (c^kc^r, c^kc^a, c^dc^r, c^dc^a), silvering is rarely expressed at all as scattered white hairs but merely as dilution, especially on the belly which tends to be a very pale cream color but not actually white. There is believed to be a real interaction effect with the lower combinations of the c-series. There are also minor interactions with spotting (*ss*) as well as mere interference in the expression of the two white patterns.

There is no sex linkage of *Si* of any sort. Rather strong linkage (22 percent recombination) is indicated with locus *M* (modifier of rough fur). Tests for linkage of *Si* with *Px*, *St*, *R*, *E*, *B*, and *P* indicated random assortment. Apparently significant linkage of *Si* and *A* (Deviation 2.4 × standard error) was probably due merely to underestimation of low grade silvering on the yellow bellies of agoutis. Tests for linkage of *Si* with *S*, *F*, and *C* were inadequate but gave no indication of linkage as far as they went. Previously reported tests (not including *Si*)

have indicated random assortment among all of the other genes above except for a weak linkage between *R* and *Px* (43 percent recombination). These appear to be indicators of 11 different chromosomes, with only two cases of linkage *R-Px* and *Si-M*. The only other suggestion of linkage in the guinea pig (and this is doubtful) is between *A* and one of the components of multifactorial polydactyly.

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LINKAGE OF FRUIT SHAPE AND COLOR GENES IN CAPSICUM[1]

PETER A. PETERSON

Department of Genetics, Iowa State College, Ames, Iowa

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IN the course of some research on peppers, *Capsicum annum* (SMITH and HEISER 1951), conducted at the Citrus Experiment Station, Riverside, California, several striking, heritable fruit characters were noted. Upon further analysis, a chromosome linkage was found to exist between two immature-fruit color genes and a gene determining pepper fruit shape. In addition the fruit shape difference between the two varieties tested was found to be due to a single gene difference.

MATERIALS AND METHODS

Parent 238 was obtained from DR. P. G. SMITH of the University of California at Davis. Parents 254 and 256 are Bureau of Plant Introduction accessions No. 203, 522 and 203, 524 respectively. The Floral Gem parent was obtained from CLARENCE BROWN of San Juan Capistrano, California. Each line used in this study was uniform and this suggests a high degree of homozygosity.

The shape of ripe fruit was measured with a millimeter-ruled pair of calipers and recordings were made to the nearest millimeter. Length is measured as the linear distance from the ovary-style fusion to the ovary base (with the receptacle removed). This is called the polar diameter. The equatorial diameter is the maximum width of the ovary. The shape index is calculated by dividing the polar diameter by the equatorial diameter. For isodiametric or round fruit the shape index is one; for elongate fruit, it is more than one.

Measurements were made of ten pepper fruits from each plant, the shape index computed for each fruit, and a mean shape index obtained for each plant.

Description of parents, F_1, F_2, and backcross progeny

There are three different parents involved in the crosses that are analyzed in this report.

Parent 238: The foliage, stems, flowers and immature fruit of these plants are dark purple. This purple anthocyanin color in the fruit results from exposure to the sun, and unexposed areas such as the tissue beneath the receptacle at the basal end of the fruit are green: purple is epistatic to green in sun exposed areas. Mature fruit color is red. From the measurement of the fruit of two plants, fruit size was found to be uniform: the mean length was 1.21 ± .09 cm and width, 1.18 ± .34 cm. The fruit shape index is 1.06 ± .02 which is indicative of nearly

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isodiametric fruit. Fruits of the 238 and Floral Gem parents, the F_1 and F_2 progeny, are shown in Figure 1.

Parent 256 is similar to 238 in the purple coloration of foliage, stem, flower and fruit, but the tissue unexposed to the sun is yellow-green. Mature fruit color is

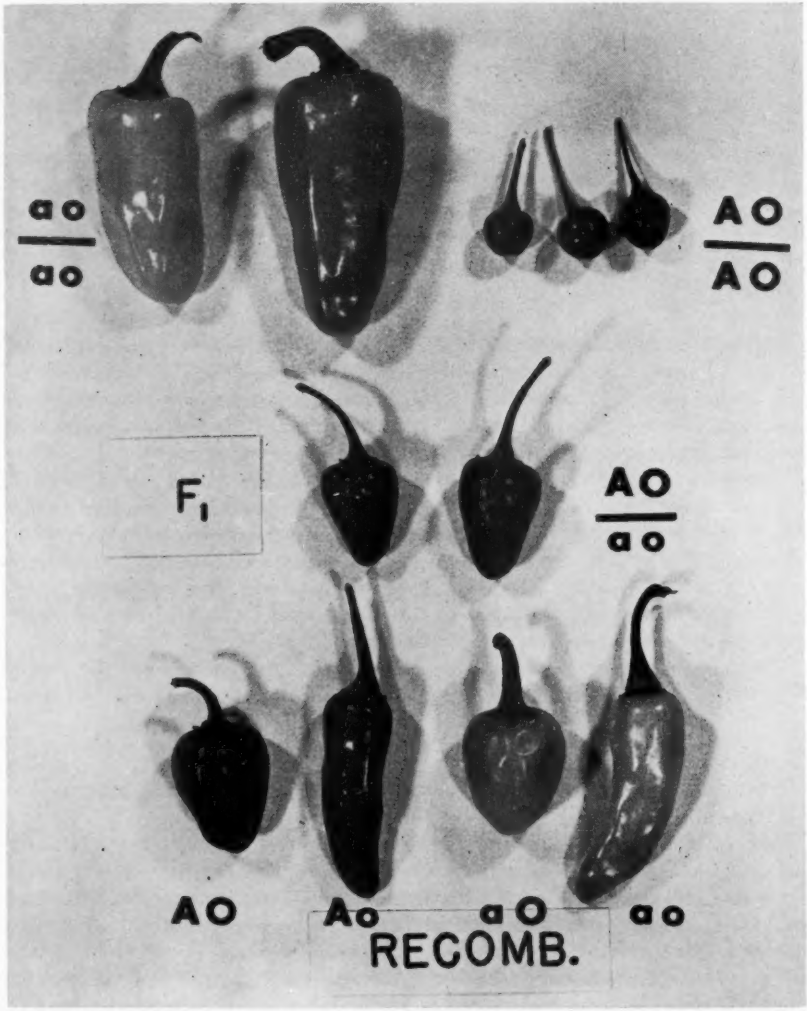

FIGURE 1.—Fruit of parents—Floral Gem and 238 (small purple). The F_1. On the bottom line, selfed progeny including parental and recombinant types—from left to right, purple round, purple long, nonpurple round, and nonpurple long.

red. Fruit size is uniform and measurement of the fruit of two plants gave an average length of 1.25 ± .11 cm and a width of 1.30 ± .23 cm. The shape index is .96 ± .02: this fruit is more oblate. Parent 254 is also purple and is similar to 256 except in fruit shape. The fruit of 254 is elongate. Purple fruit color in these purple families is associated with purple flower color (petals, style, filament and anthers). Some observed exceptions such as nonpurple style color could not be confirmed with F_3 tests.

The Floral Gem Parent has light green foliage and stems. The flowers have white petals, colorless filaments and styles and light blue anthers. The fruit is yellow when immature but turns red upon maturation. The fruit averaged 5.23 ± .51 cm in length and 2.44 ± .26 cm in width. Although fruit size was variable, the shape index was uniform, averaging 2.14 ± .08 which was twice that of either of the purple parents.

The F_1 generation: The F_1 plants from crosses of Floral Gem with parent 238 are lighter purple than parent 238 plants, but the F_1 fruit is a dark purple like the fruit of parent 238. Fruit size in the F_1 is intermediate between the two parents. (In this paper fruit size will not be evaluated.) The shape index of the F_1 fruit (from this Floral Gem × 238 cross) is uniform and averages 1.28 ± .22. The nonpurple immature fruit color is green.

The fruit of the F_1 from the Floral Gem × 256 cross is more spherical with the shape index averaging 1.22 ± .22. The nonpurple immature fruit color in plants resulting from this cross is yellow-green. The purple coloration of fruit and foliage is similar to that found in the F_1 plants of the 238 × Floral Gem cross except that the seedlings in this case are green.

The backcross and F_2 generations

Inheritance of purple and nonpurple fruit color: IKENO (1913) observed many different shades of purple plant coloration in the F_2 of the cross—purple by nonpurple. By combining all purples, he obtained a 3 purple: 1 nonpurple F_2 ratio and postulated a monofactorial difference in purple color inheritance. Although DESHPANDE (1933) also observed a 3 purple: 1 nonpurple F_2 ratio in a purple by nonpurple cross, he postulated the presence of a second locus to account for the various grades of purple.

A similarly wide range of purple coloration was noted in the foliage of the F_2 progeny of crosses of Floral Gem × 238 and 256. In fruit color classification, however, there is a clear and nonintergrading distinction between purple and nonpurple. In using only fruit color as an indicator of the purple gene, an F_2 ratio of 3 purple: 1 nonpurple is obtained and a 1 purple: 1 nonpurple ratio results from the testcross of the F_1 with Floral Gem (Table 1). (According to DESHPANDE, the purple gene is designated *A*. *AA* and *Aa* fruit are purple and *aa*, nonpurple).

Inheritance of green and yellow immature fruit color: ODLAND and PORTER (1938) observed a 15 green (various shades): 1 sulphur-white ratio in the F_2 of a cross between the varieties Ornamental (sulphur-white) and Oshkosh (cedar-green). They concluded that two pairs of alleles conditioned immature fruit color

TABLE 1

Segregating progeny from the cross AO/ao × ao/ao

Origin of *AO* chromosome	*AO*	*Ao*	*aO*	*ao*	Total	Percent recombination
			Linkage between *A* and *O* Pooled data from six backcrosses			
238	138	5	13	120	276	..
256	112	7	10	129	258	..
Total	250	12	23	249	534	6.5

Testcross segregation—*A*=262, *a*=272; *O*=273, *o*=261.

TABLE 2

Segregating progeny from the cross $AG_1/ag_1 \times ag_1/ag_1$

Origin of AG_1 chromosome	AG_1	Ag_1	aG_1	ag_1	Total	Percent recombination
			Linkage between *A* and G_1 Pooled data from four testcrosses			
254	61	10	18	66	155	....
256	49	11	19	47	126	....
Total	110	21	37	113	281	20.64

Testcross segregation—A=131, *a*=150; G_1=147, g_1=134.

in this cross: $G_1G_1G_2G_2$ represents cedar-green, $G_1G_1g_2g_2$ and $g_1g_1G_2G_2$, lettuce-green and $g_1g_1g_2g_2$, sulphur-white.

In the backcross of the yellow-green F_1 (256 × Floral Gem) by Floral Gem a 1 yellow-green:1 yellow ratio is obtained (Table 2). (In these crosses no distinction was made between yellow and sulphur-white: the yellow-green type is distinctive). Further backcrosses of yellow-green by yellow give a similar 1 yellow-green:1 yellow ratio.

The F_1 fruit from the Floral Gem by 238 cross is green but in further testcrossing with Floral Gem, a yellow-green type was isolated. In testcrosses of this yellow-green fruit type by Floral Gem a 1 yellow-green:1 yellow ratio was obtained (Tables 2 and 3). The 1 yellow-green:1 yellow testcross ratio indicates that the yellow-green fruit are heterozygous for one of the *G* loci and can therefore be designated $G_1g_1g_2g_2$. With reference to ODLAND and PORTER's symbols for green and yellow fruit color inheritance, the 256 parent is $G_1G_1g_2g_2$, parent 238 is $G_1G_1G_2G_2$ and the Floral Gem parent is $g_1g_1g_2g_2$. The yellow-green fruit color results from the segregation of G_1 or G_2 in the $G_1g_1G_2g_2 \times g_1g_1g_2g_2$ testcrosses.

Round and elongate shape: In a cross between round (oblate) and elongate fruit type, KAISER (1935) observed a monofactorial segregation for fruit shape in the F_2. KHAMBANANDA also postulated a major gene for fruit shape based on trimodal logarithmic distributions. In these two cases incomplete dominance of

the oblate type results in a distinction between the oblate homozygote, *OO*, the heterozygote, *Oo*, and the elongate, *oo*.

In all the crosses between the purple and Floral Gem parents which this author has observed, there are two and only two shapes segregating (Figures 1 and 2). The round have a shape index of approximately 1 and the elongate, which are twice as long as wide, have a shape index of 2 (Figure 3). In the F_2 of the 238 × Floral Gem cross, a 3 round:1 elongate ratio is observed (Figure 3 D). In the backcross of the F_1 to the Floral Gem parent, a 1 round:1 elongate ratio results among the testcross progeny (Table 2). In both the F_2 and testcross progenies the two classes are distinct and nonoverlapping (Figure 3 E). In this case, therefore, a major gene, *O* (round), is completely dominant to its recessive allele, *o* (elongate). (The round fruit shape is designated *OO* or *Oo* and the elongate as *oo*).

The slight differences between the fruit shape indices of the purple parents, 238 and 256, are also evident among their testcross progeny. (Figures 3A and 3C versus Figure 3F).

From the above data it is therefore possible to represent the genotypes of the four parents under consideration as follows: 238—$AAOOG_1G_1G_2G_2$, 256—

TABLE 3

Segregating progeny from the cross $OG_1/og_1 \times og_1/og_1$

Linkage between *O* and G_1						
Origin of OG_1 chromosome	OG_1	Og_1	oG_1	og_1	Total	Percent recombination
256	85	15	19	79	198	18.8

Testcross segregation—$O=100$, $o=98$, $G_1=104$, $g_1=94$.

TABLE 4

Segregating progeny from three point linkage test in cross $\frac{AOG_1}{aog_1} \times \frac{aog_1}{aog_1}$

(Pooled data from nine backcrosses)

Origin of AOG_1 chromosome	AOG_1	AOg_1	AoG_1	Aog_1	aOG_1	aOg_1	aoG_1	aog_1	Total
238	85	10	1	3	10	3	24	63	...
256	34	7	1	2	1	2	9	41	...
Total	119	17	2	5	11	5	33	104	296

Recombination		Testcross segregation
A O	OG_1	
2	17	$A=143$
5	2	$a=153$
11	5	$O=152$
5	33	$o=144$
—	—	$G_1=165$
23	57	$g_1=131$
Percent 7.77	19.25	

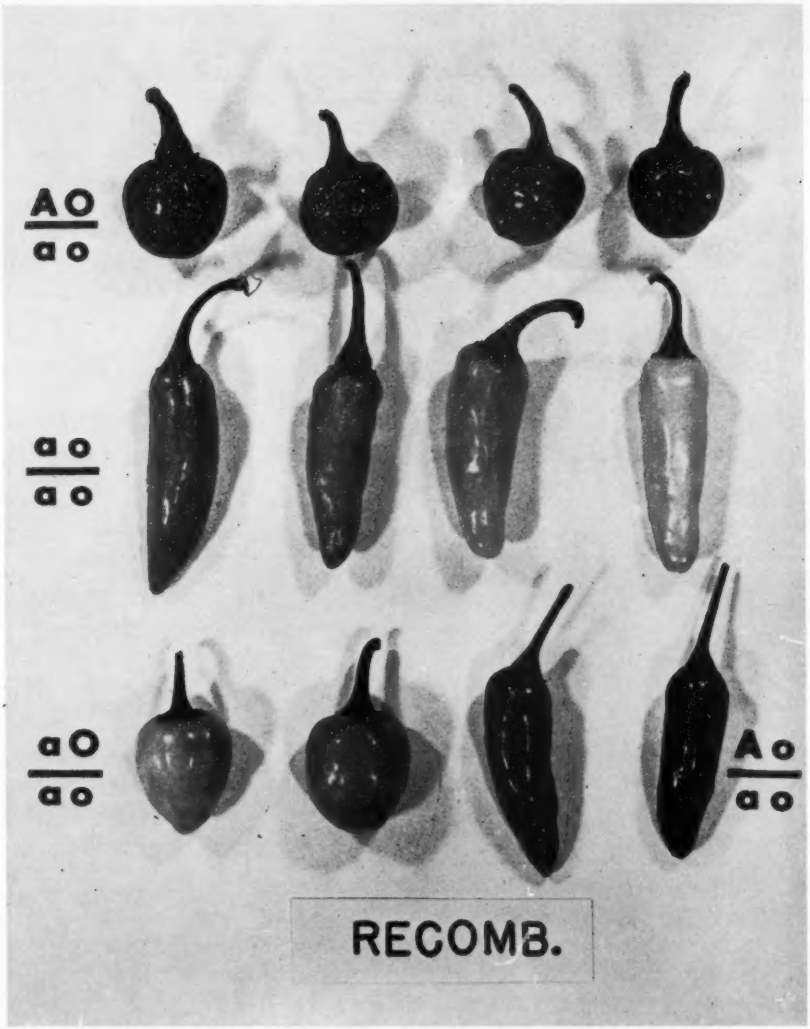

FIGURE 2.—Testcross progeny of Floral Gem and 256. First and second row—parental types. Top row—purple round *AO/ao*; second row—nonpurple long *ao/ao*. Third row— the recombinants nonpurple round and purple long. In the second row three yellow-green and one yellow (on the right). Third row left to right, round, yellow and round, yellow-green.

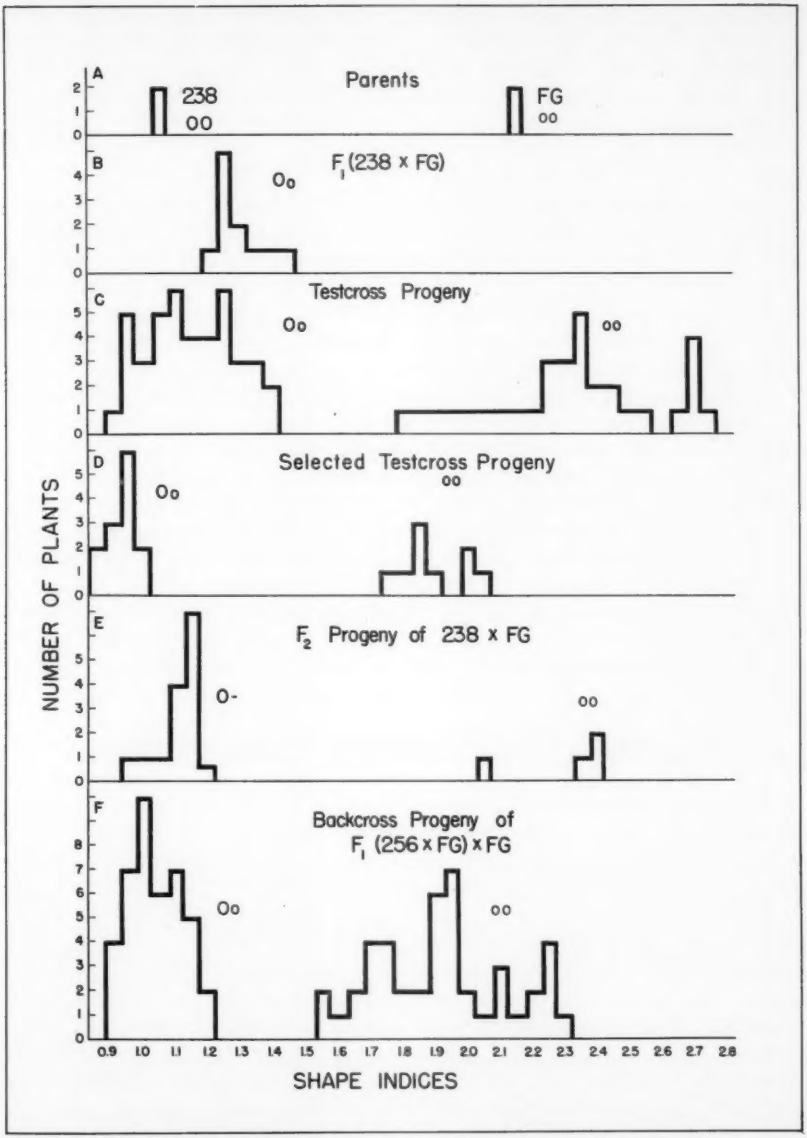

FIGURE 3.—Frequency distribution of fruit shape indices of parents (A), F_1 (B), progeny of testcross *Oo* × *oo* (C), testcross progeny of a selected *Oo* (D) and a self of *Oo* (E) from the original cross of 238 × Floral Gem (FG). In (F), testcross progeny from 256 × Floral Gem. Each value represents the average shape index of ten fruits from a plant.

AAOOG$_1$G$_1$g$_2$g$_2$, Floral Gem—*aaoog$_1$g$_1$g$_2$g$_2$*, 254—*AAooG$_1$G$_1$g$_2$g$_2$*. The purple F_1 plants from the cross 256 × Floral Gem would be *AaOoG$_1$g$_1$g$_2$g$_2$* and those from the cross 238 × Floral Gem would be *AaOoG$_1$g$_1$G$_2$g$_2$*. Further testcrosses of the 238 × Floral Gem F_1 yield a plant with round, purple, yellow-green fruit—*AaOoG$_1$g$_1$g$_2$g$_2$* or *AaOog$_1$g$_1$G$_2$g$_2$*.

Linkage of loci A, O, *and* G_1

A *and* O: In the analysis of testcross progeny of the two different purple F_1's (256 × Floral Gem and 238 × Floral Gem) by Floral Gem, it was observed that with some exception all the round fruit are purple and all the elongate fruit are nonpurple. Pooled data from numerous testcross progenies are presented in Table 1 (all crosses are in coupling). The two loci *A* and *O* are linked and 6.5 percent crossing over occurs between them. The segregation of purple *vs.* nonpurple and round *vs.* elongate is clearly monofactorial.

A *and* G_1: It was also observed that most of the nonpurple fruit are yellow and most of the purple fruit are yellow-green. Pooled data, including other purple parents that did not have the *O* gene, are presented in Table 2. *A* and G_1 are linked and 20.64 percent crossing over occurs between them.

O *and* G_1: Using the round, nonpurple recombinant, OG_1/og_1, obtained in the testcross progeny as well as AOG_1/aog_1, it was found that the percent of recombination between *O* and G_1 is 18.8 percent (Table 3). These data indicate that *A*, *O* and G_1 are on the same chromosome and in this linear order.

Further verification of this linkage order was obtained from three point testcross data (Table 4). These tests clearly show the order of genes to be *A-O-G$_1$* and the provisional map of this chromosome is as follows:

DISCUSSION

The most intensive analysis of genetic characters in Capsicum was made by DESHPANDE (1933). He postulated three instances of linkage all of which seem questionable. He calculated 44 percent crossing over between the *A* (purple immature fruit) and *R* (red mature fruit) genes. This result, however, was obtained from F_2 data and could easily represent the expected frequency based upon independent assortment. (In the author's experiments to test *A-O-G* linkage with other genes, the *R* gene appears to be independent.) In DESHPANDE's second case of linkage, also obtained from F_2 progeny, three percent crossing over was postulated between the genes for thick fruit base and calyx not enclosing the fruit base. It is more likely that these two characteristics are interdependent one upon the other. The third case involved the genes for fruit length and fruit position. Fruit length, however, did not segregate as a single gene. Moreover, it is possible that the longer fruit becomes pendent as a consequence of increased length while the

shorter fruit remains erect. DESHPANDE also indicated that shorter fruit are more often associated with purple color. He did not, however, give any linkage data on this nor did he consider shape. (This does agree, however, with the linkage reported here in that the longer fruit, those with a shape index of 2, are non-purple).

HOLMES (1934) found no linkage between the *L* gene (localized necrosis to tobacco mosaic virus) and six other genes including *A* (purple), *R* (red mature fruit), *S* (smooth stem), *D* (pointed apex), *P* (pendent fruit position), and *M* (pungent fruit). HAGIWARA and OOMURA (1947) observed 20 to 24 percent recombination between the genes for purple style and purple pistil and approximately 15 percent recombination between pendent peduncle and a gene for variegated leaf.

Control of fruit shape

In this paper a major dominant gene that controls fruit shape in peppers is described. This evidence is derived from an analysis of F_2 and testcross progenies. In these progenies the fruit shape indices are distinct and nonoverlapping (Figure 3 C,D,E,F). In the testcross (*Oo* × *oo*) progenies the heterozygotes are round with a shape index of approximately 1 and the homozygous recessives are elongate with a shape index of approximately 2. In the selfed progeny the homozygotes (*OO*) and heterozygotes (*Oo*) are round and their shape index is approximately 1 (Figure 3 E). This similarity between *OO* and *Oo* in the shape index analysis of F_2 progenies indicates that the *O* allele is completely dominant.

Such a gene controlling fruit shape in peppers was hypothesized by KAISER (1935) and KHAMBONANDA (1950). In their studies, however, trimodal curves of F_2 populations indicated only partial dominance of the oblate type. (It is not known whether the *O* gene of the crosses discussed in this paper is similar to the shape controlling gene described by KAISER and KHAMBONANDA).

Although a single gene that determines fruit shape is demonstrated in the crosses reported here, there appear to be other genes which also govern fruit shape. This is evident in studying backcross progeny of crosses of the 238 parent with longer fruited types (shape index = +4). These result in progeny of intermediate fruit shape and in addition the backcross progeny segregate less sharply. Similar results were obtained by DALE (1929). His data show a continuous distribution among the progeny of peppers with parents of very dissimilar fruit types, and he concluded therefore that fruit length inheritance is based on genes with proportionate effects. (In DALE's crosses, the extremes between the parents probably represent more than one gene difference).

The difference in shape index between the 238 and 256 parental types and the distribution of shape indices in their progeny (Figure 3 C, F) is due to segregating modifiers and not to a difference in the particular alleles of the original parents. This conclusion is based on a study of a derivative of the backcross progeny of the 238 allele. After two successive backcrosses, the segregation of round and elongate types is sharper and, as the curves (Figure 3 D) indicate, there is a definite shift to the left resulting in a curve similar to that derived from

the progeny of the 256 allele. This probably results from the segregation of modifiers that cause elongation of the fruit.

A number of cases have been reported of genes governing fruit shape in other plants. SINNOTT (1927) found that two pairs of alleles differentiate the disk from the elongate fruit type in Cucurbita. In Lycopersicum, LINDSTROM (1927) analyzed a major incompletely dominant gene controlling fruit shape. In *Solanum melongena*, GOTOH (1954) hypothesized that major genes control fruit shape.

The control of shape in terms of gene action was considered by SINNOTT (1935) and KAISER (1935) working with the squash and pepper, respectively. They concluded that shape differences result from differences in genes which control the relative rates of growth between the polar and equatorial dimensions. This conclusion was based on data which showed a sharper segregation of shape indices than of dimensional traits among F_2 progeny. Although a clear bimodality was evident for length in both the squash and pepper, the segregation was not so sharp as for shape. A smooth curve was obtained for width. From this the authors concluded that there is a gene governing shape and that therefore there is no direct genic control upon individual dimensions (length and width). They further concluded that any apparent segregation for length was the indirect result of "interactions between genes controlling dimensional relationships". (KAISER 1935).

In the studies reported here the lengths and widths of the two genotypes, *Oo* (round), and *oo* (elongate) from the cross *Oo* × *oo*, are plotted and analyzed separately. Using this type of analysis, there is observed a distinct though slightly overlapping segregation for the fruit lengths of the two fruit types (Figure 4). This difference between the mean lengths of the elongate and round fruit is highly significant—$P = < .01$ (Table 5). The widths of the elongate and round fruit overlap to a considerable extent. The difference between the means of the two fruit widths, however, is significant—$P = < .05$ (Table 6). There is evident also a negative correlation between length and width in the backcross progeny. This would be expected since the elongate fruit becomes narrower as it elongates while the width of the round fruit remains unaffected. (SINNOTT 1935, found a similar negative correlation between length and width among the F_2 progeny of

TABLE 5

Mean lengths and variances of round and elongate fruit from the cross Oo × oo

	n	dt.	Mean length	Sum of squares
Round *Oo*	35	34	2.275	9.65
Elongate *oo*	32	31	3.6765	7.97
		65	diff. = 1.4015 $Sx^2 =$	17.62
		pooled variance	$= s^2 =$ 17.62/65 =	.2710
		$s(\overline{X}_1 - \overline{X}_2)$	= .127	
		t	= 1.4015/.127 = 11.03	(P=<.01)*

* Significantly different from 0 cm difference. P=<.01.

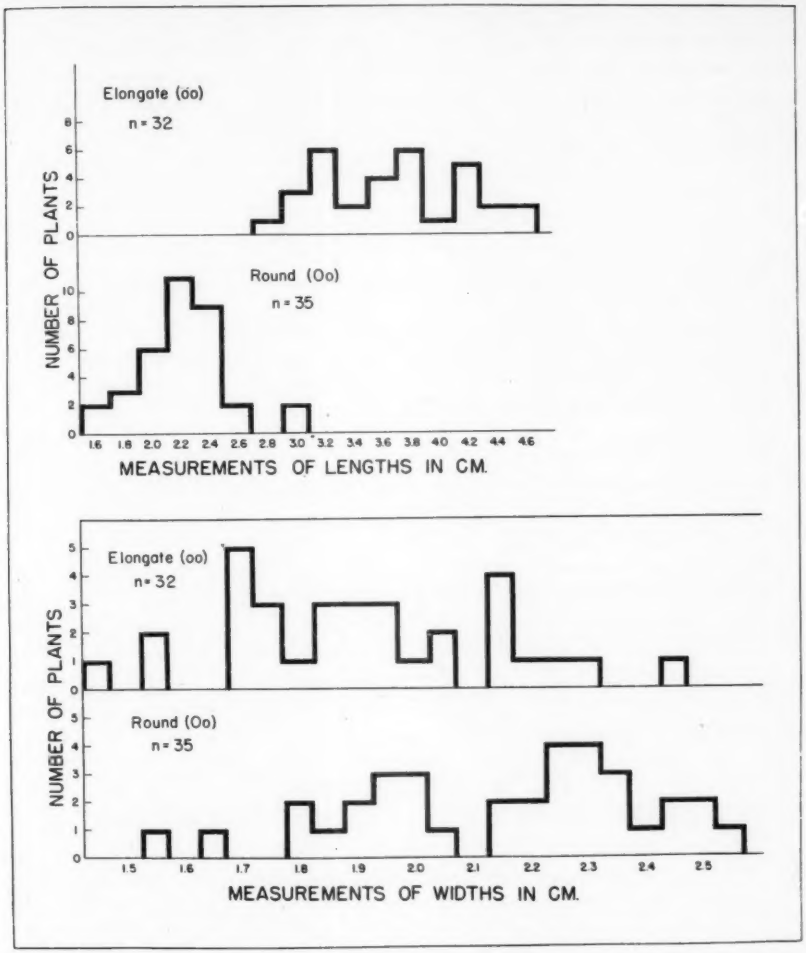
Elongate (oo)
n = 32
Round (Oo)
n = 35
NUMBER OF PLANTS
1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 3.6 3.8 4.0 4.2 4.4 4.6
MEASUREMENTS OF LENGTHS IN CM.
Elongate (oo)
n = 32
Round (Oo)
n = 35
NUMBER OF PLANTS
1.5 1.6 1.7 1.8 1.9 2.0 2.1 2.2 2.3 2.4 2.5
MEASUREMENTS OF WIDTHS IN CM.

FIGURE 4.—Frequency distribution of measurements of lengths and widths of mature fruit of backcross progeny from the cross of F_1 (256 × Floral Gem) - *Oo* × Floral Gem - *oo*. Each value represents the average measure of ten fruits from a plant.

squashes.) Therefore a change in shape can be explained by postulating a change in length which causes a coincident change in width. Such is the case with a rubber band: if stretched, the width coincidentally narrows. Thus only a single action is necessary to cause such a difference in shape.

From these results—the clear segregation for fruit length and the negative

TABLE 6

Mean widths and variances of round and elongate fruit from the cross Oo × oo

	n	df.	Mean width	Sum of squares
Round *Oo*	35	34	2.10 cm.	6.3082
Elongate *oo*	32	31	1.911	1.6618
		65	diff. = .189	$Sx^2 = 7.9700$
		pooled variance	$= s^2 = 7.9700/65 = .1226$	
		$s(\overline{X}_1 - \overline{X}_2)$	= .0856	
		t	= .189/.0856 = 2.207	P=<.05*

* Significantly different from 0 cm difference. P=<.05.

correlation between length and width—it is postulated that the factor controlling linear dimension is the major influence in governing shape. On this basis, the *O* gene controls shape by controlling length.

This is opposed to SINNOTT's and KAISER's views on shape control which are based upon the belief that shape is controlled by genes which coordinate many pathways of growth. Such a multifunctional role for genes is not consistent with present day views which hypothesize that genes control single steps and one action (HOROWITZ 1956; HOROWITZ and FLING 1956).

SINNOTT (1931) also established that size of fruit is independent of shape. This is evident in the pepper crosses reported here. Although the size of the round fruit of the backcross progeny is nearly double the fruit size of parent 238 (Figure 1) the shape index of 1 is maintained in the presence of the *O* gene.

In all these cases (tomato, squash, eggplant and pepper) it is the dominant alleles that tend to flatten the fruit while elongate fruit develop in the presence of the recessive alleles.

SUMMARY

1. Linkage of three loci in peppers is here reported. These include the genes *A* (purple versus nonpurple immature fruit color), *O* (round versus elongate fruit shape) and G_1 (yellow-green versus yellow immature fruit color). The linear order and the map distances of the genes is as follows: *A*—6.5—*O*—18.8—G_1.
2. Among the progeny of selfed and testcrossed heterozygotes, *Oo*, the two fruit shapes—round and elongate—are distinct and nonintergrading.
3. The *O* gene governing fruit shape is completely dominant.
4. Although the *O* gene is completely dominant, other genetic modifiers also govern fruit shape.
5. The *O* gene controls shape independent of size.
6. Fruit shape is postulated to depend primarily on fruit length.
7. The role of genes in the control of shape is discussed.

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NON RANDOM ASSORTMENT OF NON-HOMOLOGOUS CHROMOSOMES IN DROSOPHILA MELANOGASTER[1]

RHODA F. GRELL

Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena, California

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NON-HOMOLOGOUS chromosomes assort independently at meiosis. This principle has been repeatedly confirmed by genetical and cytological experiments. Under abnormal or special conditions exceptions to this principle have been reported (GERSHENSON 1940; LONGLEY 1944; MITCHIE and WALLACE 1953; SANDLER and NOVITSKI 1956).

In the experiments described in this paper a markedly non random assortment of non-homologous chromosomes of *Drosophila melanogaster* has been discovered. A normal Y chromosome and a normal fourth chromosome have been found under certain conditions to segregate from each other with a frequency as high as 92 percent. The details of the experiments and the assortment results are presented below.

MATERIALS AND METHODS

Non random assortment of the sex chromosomes and the fourth chromosomes has been studied principally in XXY females heterozygous for a translocation between the third and fourth chromosomes. Such females will be referred to as Type A females. The extra Y chromosome of Type A females was derived from a Canton-S stock and will be designated Y^{C-S}. The principal translocation used in Type A females was obtained from E. B. LEWIS and is known as T(3;4)86D (GRELL 1956). It is a reciprocal translocation between the right arm of the third chromosome and the basal portion of the right arm of the fourth chromosome. Salivary gland chromosome analysis by LEWIS shows a break in the third chromosome just after 86D1–2 and a break in the fourth chromosome at 101F. The stock of this translocation and the stock of T(3;4)88B, which is described below, were cleared of extra fourth chromosomes prior to the start of the experiments. In order to select against triplo-4 flies in preliminary crosses involving either translocation, all free fourth chromosomes present in such crosses were marked with dominants.

Type A females were obtained among the progeny of the following cross (Cross No. 1):

$$\frac{y^2 v}{\text{FM3, } y^{31d} sc^8 dm\ B\ l};\ Y^{C-S};\ \frac{In(2L)Cy,Cy + In(2R)bw^{VDe1},\ bw^{VDe1}}{bw};\ \frac{ci\ sv^n}{ci\ sv^n}\ \text{♀} \times$$

$$y^2 v;\ Y^{C-S};\ Y^{C-S};\ \frac{In(2L)Cy,Cy + In(2R)bw^{VDe1},\ bw^{VDe1}}{bw};\ \frac{\text{T(3;4)86D, } bx^{34e} e^4}{+;\ ey^D}\ \text{♂}$$

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where FM3 symbolizes a complex X chromosome balancer known as "First Multiple 3" GRELL. (The FM3 balancer (GRELL 1954) was derived from X-irradiation of males carrying two X chromosome inversions scute-8(sc^8) and delta-49(dl-49), and the mutants y^{31d}, *dm* and *B*. It carries a lethal of unknown locus. Salivary gland chromosome analysis (LEWIS 1954) shows three new breakage points in regions 3EF, 16AB and in the proximal heterochromatin (19F–20) superimposed on those of sc^8 and delta-49).

The presence in the parents of Cross No. 1 of a chromosomal rearrangement associated with dominant brown variegation, namely $In(2R)bw^{VDe1}$ served as the means for identifying the presence of the extra Y chromosome in those parents. It is well known that such variegation is virtually completely suppressed in the XXY female or the XYY male (GOWEN and GAY 1933; SCHULTZ 1936). The reasons for the other markers will become apparent below.

The non-Bar, non-Curly, non-eyeless^D virgin daughters of Cross No. 1 were individually mated to XY males heterozygous for bw^{VDe1}. This constitutes Cross No. 2:

$$\frac{y^2v}{y^2v};\ (Y^{c-s})\,(Y^{c-s});\ \frac{bw}{bw};\ \frac{T(3;4)86D,\ bx^{34e}e^4}{+\ ;\ ci\ sv^6}\ \varphi \times v\ ;\ \frac{In(2R)bw^{VDe1},bw^{VDe1}}{SM1,\ al^2Cy\ sp^2}\ \delta$$

The number of Y chromosomes present in the parental females of Cross No. 2 is expected to vary from none to two. Females which carry one extra Y are Type A; females which carry no Y will be referred to as Type B and females which carry two extra Y's will be referred to as Type C.

The method of determining the number of Y chromosomes in the parental females of Cross No. 2 was to observe the proportion of regular bw^{VDe1} (non-Curly) progeny which showed suppression of the brown variegation.

If the parental female of Cross No. 2 is Type A (XXY^{c-s}) then the bw^{VDe1} regular progeny are expected to consist of approximately equal numbers of suppressed and unsuppressed types. That is, approximately one-half of the regular progeny of XXY females carry an extra Y chromosome.

If the parental female of Cross No. 2 is Type B (XX) then all of the bw^{VDe1} regular progeny are expected to have unsuppressed brown variegation. That is, there will normally be no extra Y's in the progeny of XX females.

If the parental female of Cross No. 2 is Type C ($XXY^{c-s}Y^{c-s}$) then most of the bw^{VDe1} regular progeny are expected to have suppressed brown variegation. This follows from STERN's analysis (1929) which showed that approximately 98 percent of the regular progeny of XXYY females carry an extra Y chromosome.

The detection of the suppression of variegation is facilitated by the presence of the mutants vermilion (v) and brown (bw). Thus, $\frac{v}{v};\ \frac{bw^{VDe1}}{bw}$ females or v; Y; $\frac{bw^{VDe1}}{bw}$ males have nearly white eyes, whereas the addition of an extra Y chromosome to either of these genotypes gives a bright red color virtually indistinguishable from that of v. For this reason, v and bw were introduced into Crosses Nos. 1 and 2.

In order to follow the disjunction of the X chromosomes in the progeny of Cross No. 2, the parental female was made homozygous for the body color mutant, yellow^2. The regular progeny of Cross No. 2 thus consist of y^+ females and y^2 males, whereas the nondisjunctional progeny consist of y^2 females and y^+ males.

Since the parental females of Cross No. 2 were heterozygous for a translocation involving the third and fourth chromosomes, there will be an appreciable number of offspring nondisjunctional for the fourth chromosomes. That is, the progeny of Cross No. 2 will be composed of haplo-4 and triplo-4 flies as well as the regular diplo-4 flies.

Haplo-4 types, which are late-hatching, were scored directly on the basis of their characteristic complex of dominant effects (Minute bristles, small body size, pale body color, pronounced trident pattern and spread wings).

As triplo-4 flies are not noticeably different phenotypically from diplo-4 flies, it is necessary to distinguish these types from one another by progeny tests. The tester stock employed is *Ubx*/+; *M-4*/ey^D where *Ubx* is Ultra-bithorax; *M-4* is Minute-4; and ey^D is eyeless-Dominant. Samples of 105 suppressed and 391 unsuppressed bw^{vDel} non-haplo-4 progeny from Cross No. 2 were selected for the test. Such flies are expected to consist of three classes,

1. Diplo-4 flies not carrying T(3;4)86D.
2. Diplo-4 flies heterozygous for T(3;4)86D.
3. Triplo-4 flies heterozygous for T(3;4)86D and carrying two free fourth chromosomes.

When a Class 1 fly is mated to the tester stock, it is expected to produce only diplo-4 flies. Therefore all the non-eyeless-Dominant offspring are expected to be Minute-4 in phenotype.

Class 2 flies when mated to the tester stock, although diplo-IV in composition, are expected to produce some triplo-4 offspring as the result of nondisjunction induced by T(3;4)86D. Non-eyeless-Dominant flies that are triplo-4 are easily recognized because they have complete suppression of the Minute-4 phenotype. The problem, then, is to distinguish Class 2 flies from Class 3 flies since the latter will also produce triplo-4 progeny. This can be done, as will be shown below, by first identifying the translocation-bearing progeny of Class 2 and Class 3 flies. The presence of the dominant marker *Ubx* in the tester stock serves this purpose. Translocation (3;4)86D carries a rarely separable marker, bithorax^{34e} (bx^{34e}) which is a recessive pseudoallele of *Ubx*. The "trans" form of the double heterozygote *bx* + / + *Ubx* has a large wing-like haltere which is clearly distinguishable from ++ / + *Ubx*. Moreover, if a rearrangement is present in the vicinity of the bithorax mutants, a further modification of the trans-type position effect occurs. This consists of the development of a hairy band of tissue across the mesonotum and is known as the "transvection effect" (LEWIS 1954). The presence of this extra band of tissue indicates that the marker bx^{34e} is still associated with the translocation.

Class 2 and Class 3 flies may now be distinguished from each other by examining their translocation-bearing progeny. If the parent is Class 2 (diplo-4), a

maximum of 50 percent of the progeny which carry the translocation are expected to carry two free fourth chromosomes. Thus a maximum of 50 percent of the translocation-bearing progeny are expected to show suppression of the Minute phenotype. If the parent is Class 3 (triplo-4), 90 percent or more of the progeny which carry the translocation are expected to carry two free fourth chromosomes. This is based on STURTEVANT's observation (1936) that the two normal fourth chromosomes are recovered together in about five percent of the progeny of females which carry an X-4 duplication in addition to two normal fourth chromosomes. In the case of a Class 3 parent, over 90 percent of the translocation-bearing progeny are expected to show and did show suppression of the Minute phenotype.

In order to follow the segregation of the fourth chromosomes more directly, i.e., without the necessity of progeny testing, two additional experiments were devised. The first of these (Cross No. 3) is identical with Cross No. 2 except that the free fourth chromosome of the female parent carries the dominant marker cubitus-interruptus-Dominant (ci^D) instead of *ci* sv^n. The absence of the dominant marker in one class of progeny provides a means of directly identifying it as the diplo-4, translocation-bearing class.

In the second experiment (Cross No. 4) the parental female carries a dominantly marked translocation as well as a free fourth chromosome marked with ci^D. It was easier to use a different translocation already containing a dominant marker than to attempt to introduce a dominant marker into T(3;4)86D. The translocation chosen was T(3;4)88B which carries the virtually inseparable dominant marker *Ubx*. Salivary gland chromosome analysis of T(3;4)88B by LEWIS shows a break in 3R after 88B and a break in 4R at 101. Although in other respects the genotypes of the parents in Cross No. 4 are identical with those in Cross No. 2, the presence of the two dominant markers enables one to follow results of the segregation of the fourth chromosomes in all classes of progeny.

The female parent which was used in the final cross (Cross No. 5) was not of Type A. Instead, a female (Type D) was constructed which was homozygous for T(3;4)86D, $bx^{34e}e^4$ and which carried in addition a free fourth chromosome marked with ci^D or sparkling Cataract (spa^{Cat}). Otherwise the genotypes of the parents were again identical with those used in Cross No. 2. All of the progeny from Cross No. 5 are heterozygous for T(3;4)86D and the segregation of the free fourth chromosome is easily followed by observing which progeny are ci^D or spa^{Cat} (triplo-4) and which progeny are non-ci^D or non-spa^{Cat} (diplo-4).

RESULTS AND ANALYSIS

If the assortment of the sex chromosomes and the fourth chromosomes are independent of each other in Type A (XXY;heterozygous for a 3–4 translocation) females, suppressed (extra Y) and unsuppressed (no extra Y) progeny are expected to appear in equal numbers among each of the two products of regular disjunction and each of the two products of nondisjunction of the fourth chromosomes. The progeny of Type A females from Cross No. 4 will be considered first since all of the classes of progeny are phenotypically identifiable. An examination

of Cross No. 4 progeny (Table 3) shows that the ratio of suppressed to unsuppressed classes in the haplo-4 group of flies is 53:1 and that the ratio in the triplo-4 flies is 1:62. Both ratios represent highly significant deviations from the expected 1:1 ratio. Furthermore, the ratio of suppressed to unsuppressed classes in the diplo-4, nontranslocation flies is 1:1.6. This is a statistically significant deviation from the expected 1:1 ratio ($P < .002$). The ratio of suppressed to unsuppressed classes among the diplo-4, translocation flies shows no significant deviation from a 1:1 ratio.

The progeny of Type A females from Cross No. 2 (Table 3) will be considered next. In the haplo-4 group, the only group in which the number of fourth chromosomes can be determined directly, suppressed and unsuppressed classes are found in the ratio of 17:1. The proportions of flies in the other three groups were determined by progeny tests. All of the fertile samples were successfully classified according to the criteria outlined under Materials and Methods. The results of the tests (Table 3) show that the ratio of suppressed to unsuppressed classes in the triplo-4 group is 1:24; that the ratio of suppressed to unsuppressed classes in the diplo-4, nontranslocation group is 1:2.4; and that the ratio of suppressed to unsuppressed classes in the diplo-4, translocation group is 1.8:1. Each ratio represents a statistically highly significant deviation from 1:1.

Lastly, the progeny of Type A females from Cross No. 3 (Table 3) contain two groups in which the fourth chromosome content can be directly determined. In the first of these, the haplo-4 group, suppressed and unsuppressed classes are found in the ratio of 22:1. In the second or diplo-4, translocation-bearing group, suppressed and unsuppressed classes are found in the ratio of 2:1. The third or triplo-4 group is indistinguishable phenotypically from the fourth or non-translocation diplo-4 group. Among the combined third and fourth groups, however, the suppressed and unsuppressed classes are phenotypically distinguishable and have the ratio of 1:5.6. For comparison, the ratio of the suppressed and unsuppressed classes when the latter two groups from Cross No. 2 are summed is 1:4.9 and similarly for Cross No. 4, the corresponding ratio is 1:3.8.

The data from Crosses Nos. 2, 3, and 4 are rearranged in Table 4 with respect to the types of segregation involved and the classes of progeny that result from each segregation type. There are four types of segregation each of which produces two complementary classes which should be numerically equal if the viability of each class is the same. Type-I and Type-II segregations are defined as those nondisjunctional for the fourth chromosomes. The difference between them is that in Type-I the extra Y chromosome goes to the opposite pole from the normal fourth chromosome and the translocation while in Type-II the extra Y goes to the same pole. Both segregations lead to haplo-4 and triplo-4 flies. As haplo-4's are known to have a low and erratic viability, the two complementary classes from Type-I and Type-II segregations are not expected to, and do not, appear in equal numbers. The relative frequency of Type-I and Type-II can be measured by comparing the number of haplo-4 flies from Type-I with those from Type-II or by comparing the number of triplo-4 flies from the same segregations. The frequency of

Type-I based on haplo-4 flies varies, depending on the cross, from 17 (Cross No. 2) to 53 (Cross No. 4) times the frequency of Type-II and based on triplo-4 flies from 24 (Cross No. 2) to 62 (Cross No. 4) times the frequency of Type-II. This means that from Type-I and Type-II segregations, the normal fourth chromosome and the translocation are recovered without the extra Y chromosome some 17 to 62 times as frequently as they are recovered with the extra Y.

Type-III and Type-IV segregations are defined as those arising from the regular disjunction of the fourth chromosomes. They lead to diplo-4 progeny, 50 percent of which are expected to carry the translocation and 50 percent of which are expected to carry a normal third and fourth chromosome. The distinction between Type-III and Type-IV segregation is again in the assortment of the Y chromosome which goes to the same pole as the translocation in Type III and to the same pole as the normal third and fourth chromosomes in Type-IV. The two complementary products from Type-III show a significant departure from equality in Cross No. 4 but not in Cross No. 2. The 44 percent deficiency that is observed in the extra Y, translocation-bearing class from Cross No. 4 may be due to reduced viability for this type of zygote or to a meiotic event which leads to a reduction in the number of viable extra Y, translocation-bearing gametes. Type-IV segregation produces two approximately equal complementary classes from Cross No. 2 and from Cross No. 4. Cross No. 3 does not permit a comparison of classes from Type-III or Type-IV for only one class is identified in each case.

The relative frequency of Type-III and Type-IV segregations can be measured by comparing the numbers of translocation progeny from each type or by comparing the numbers of nontranslocation progeny from each type. When the former criterion is used, Type-III is found to be 1.8 times as frequent as Type-IV segregation in Cross No. 2 and twice as frequent as Type-IV in Cross No. 3. In Cross No. 4, Type-III is only .77 times as frequent as Type-IV. The cause of the apparent inconsistency is the 44 percent deficiency of the extra Y, translocation-bearing class in Cross No. 4. A comparison of Type-III and Type-IV segregations based on the diplo-IV, nontranslocation classes shows that the frequency of Type III varies depending on the cross, from 1.6 (Cross No. 4) to 2.4 (Cross No. 2) times that of Type-IV. This means that from Type-III and Type-IV segregations, the normal fourth chromosome is recovered without the extra Y chromosome some 1.6–2.4 times as frequently as it is recovered with the extra Y chromosome and that the translocation is recovered with the extra Y chromosome some .77–2 times as frequently as it is recovered without it.

The total frequency with which the extra Y chromosome and the normal fourth chromosome are recovered together in the progeny of Type A females can be estimated for each cross by totaling the non-extra Y progeny from the cross and then determining what percent of this total is represented by flies which do not carry the normal maternal fourth chromosome. The calculations are based on the non-extra Y classes in order to eliminate the large haplo-4 extra Y bearing class whose viability is low and erratic. The small haplo-4, non-extra Y bearing class is necessarily included in the progeny from which the estimates are derived.

A viability correction of 3.0 is assigned to this class in each cross since this is the value which is needed to equalize the complementary haplo-4 and triplo-4 classes from Type-I segregation in Cross No. 2 (Table 4) where the discrepancy between them is greatest. The values for the recovery of the extra Y and fourth chromosome together are as follows:

Cross No. 2, 16.8 percent = $[(2.4 \cdot 3.0) + 34.5] \div [(2.4 \cdot 3.0) + 118 + 88 + 34.5]$
Cross No. 3, 16.2 percent = $[(5 \cdot 3.0) + 104] \div [(5 \cdot 3.0) + 616 + 104]$
Cross No. 4, 22.4 percent = $[(2 \cdot 3.0) + 83] \div [(2 \cdot 3.0) + 186 + 123 + 83]$

Since the expectation of recovery of the Y and fourth chromosome together is 50 percent if their assortment is random, the values obtained are obviously much under the expectation. Of interest is the similarity in the values (16.2 and 16.8 percent) which are obtained when the same translocation is used in different experiments (Cross No. 2 and No. 3) and the difference in value (22.4 percent) when a different translocation is used (Cross No. 4).

The frequency with which the extra Y chromosome and the translocation are recovered together in the progeny of Type A females can also be estimated for Cross No. 2 and No. 4. The values for the recovery of the Y and translocation together are as follows:

Cross No. 2, 38.5 percent = $[(2.4 \cdot 3.0) + 88] \div [(2.4 \cdot 3.0) + 118 + 88 + 34.5]$
Cross No. 4, 32.4 percent = $[(2 \cdot 3.0) + 123] \div [2 \cdot 3.0) + 83 + 123 + 186]$

The values are again certainly much under the expectation of 50 percent. On the basis of BROWN's data (1940) for a heterozygous 3–4 translocation with a break at 87E3–87F1, the nontranslocated fragment of the third chromosome pairs and disjoins regularly with the normal three. The translocated fragment of three shows a high rate of nondisjunction with the normal three. Presumably it is this fragment translocated to the four centromere which assorts non randomly with the extra Y chromosome.

The difference in the frequency of recovery of Y,4 and Y. translocation together in the two translocations which were used may be correlated with the degree to which the translocation has impaired pairing between the two fourth chromosomes. In the case of T(3;4)86D. the genetic data indicate that the free fourth chromosome assorts nearly randomly with respect to its homologue involved in the translocation so that 22 percent haplo-4, 22 percent triplo-4 and 56 percent diplo-4 progeny are produced. The observed percentage of haplo-4 flies from Type B females (XX;T(3;4)86D/ +) from Cross No. 2 (Table 1) is 8.62. An estimate of the viability of the haplo-4 class has been made by comparing the number of suppressed haplo-4 flies from Type A females, which were observed directly, with the number of complementary unsuppressed triplo-4 flies from the same segregation (Type I segregation) which were determined by progeny tests (Table 4). On this basis the viability of the haplo-4 flies appears to be approximately one-third of the triplo-4 flies and a viability correction of about 3 for the haplo-4 class is in order. The corrected value for haplo-4 flies from Type B females of Cross No. 2 is 22.05 percent ($3 \times 8.62 \div 100 + 2 \times 8.62$) indicating a nearly random assortment of the fourth chromosomes.

TABLE 1

Results from Cross No. 2. Progeny of y^2v/y^2v; bw/bw; *T(3;4)86D,* bx^{34e} $e^4/+$; ci sv^n *females which carry no Y (Type B), one Y (Type A) or two Y's (Type C) chromosomes and* v; In(2)bw^{VDe1}, bw^{VDe1}/SM1, al^2 Cy sp^2 *males*

Type of ♀ parent	Regular disjunction of X chromosomes Suppressed ♀ Diplo- or Triplo-4	♀ Haplo-4	♂ Diplo- or Triplo-4	♂ Haplo-4	Nonsuppressed ♀ Diplo- or Triplo-4	♀ Haplo-4	♂ Diplo- or Triplo-4	♂ Haplo-4
Type A (1 Y)	906	230	877	448	2,173	15	1,917	25
Type B (no Y)	...	...	...	...	733	53	733	86
Type C (2 Y)	392	27	372	58	27	0	17	0
	Primary or secondary nondisjunction of X chromosomes							
Type A (1 Y)	42	5	...	...	...	...	63	2
Type B (no Y)	3	...	...	...	...	...	3	...
Type C (2 Y)	3	...	4	...	...	...	6	...
	Percent regular progeny		Percent nondisjunctional progeny				Percent haplo-4's	
Type A (1 Y)	98.3		1.7				10.9	
Type B (no Y)	99.6		.4				8.6	
Type C (2 Y)	98.6		1.4				9.5	

In the case of T(3;4)88 *Ubx* the number of haplo-4 and triplo-4 progeny from Type B females is observed directly (Table 2). The viability of the haplo-4 class is equal to one-third of that of the triplo-4 class (26:78). The corrected sum of haplo-4 and triplo-4 flies is about 19 percent ($2 \times 78 \div 764 + 2/3 \times 78$) of the progeny. The assortment of the fourth chromosomes appears to be much more regular in this case.

TABLE 2

Results from Cross No. 4. Progeny of y^2 v/y^2 v; bw/bw; *T(3;4)88B,* Ubx/+; ci^D *females which carry no Y (Type B) or one Y (Type A) chromosome and* v; In(2)bw^{VDe1}, bw^{VDe1}/SM1, al^2 Cy sp^2 *males*

Type of ♀ parent	Regular disjunction of X chromosomes Suppressed ♀ *Ubx*	♀ ci^D	♀ *Ubx* ci^D	♀ Haplo-4	♂ *Ubx*	♂ ci^D	♂ *Ubx* ci^D	♂ Haplo-4	Nonsuppressed ♀ *Ubx*	♀ ci^D	♀ *Ubx* ci^D	♀ Haplo-4	♂ *Ubx*	♂ ci^D	♂ *Ubx* ci^D	♂ Haplo-4
Type A (1 Y)	32	41	1	41	32	37	2	64	37	61	81	1	46	62	105	1
Type B (no Y)	..	..	..	..	..	..	..	..	157	188	45	13	147	168	33	13
	Primary or secondary nondisjunction of X chromosomes															
Type A (1 Y)	1	2	0	1	..	..	..	..	..	..	..	..	4	3	0	0
Type B (no Y)	..	..	..	1	..	..	..	..	..	..	..	..	..	..	2	..
	Percent regular for X's				Percent nondisjunctional for X's								Percent Haplo-4's			
Type A (1 Y)	98.3				1.7								16.3			
Type B (no Y)	99.6				.4								3.5			

TABLE 3

Progeny of Type A females from Crosses No. 2, 3 and 4 arranged to show the assortment of the Y chromosome in the four kinds of progeny which result from regular disjunction and nondisjunction of the fourth chromosome

		Cross No. 4 T(3;4)88Ubx/+; ci^D			Cross No. 2 T(3;4)86D/+; $ci\ sv^a$			Cross No. 3 T(3;4)86D/+; ci^D		
		Y	O	Ratio Y:O	Y	O	Ratio Y:O	Y	O	Ratio Y:O
Nondisjunction of fourth chromosomes	Haplo-IV	105	2	53:1	678	40	17:1	109	5	22:1
		...	...	...	39.9†	2.4†	...	...	...	...
	Triplo-IV	3	186	1:62	(5)	(192)	1:24	...	...	...
		...	...	...	...	118*	...	...	...	...
								110	616	1:5.6
Regular disjunction of fourth chromosomes	III; IV	78	123	1:1.6	(37)	(143)	1:2.4	...	...	...
		...	...	...	...	88.1*	..	...	...	...
	Translocation	64	83	.77:1	(63)	(56)	1.8:1	204	104	2:1
		...	...	...	...	34.5*	...	...	...	...

Numbers in parenthesis indicate determination by progeny tests.
* Corrected value. Correction is necessary because the suppressed and unsuppressed flies were not progeny tested in the proportion in which they are produced. Correction factor equals 0.616 = Ratio Y:O in total sample of non-haplo-4's/Ratio Y:O in progeny tested samples.
† Number of haplo-4 flies expected to be produced in a sample containing the number of suppressed non-haplo-4 animals that were progeny tested.

TABLE 4

Progeny from Type A females from Crosses No. 2, 3 and 4 arranged with respect to the four types of segregation involved and the two complementary classes which result from each type

	Segregations nondisjunctional for 4th chromosomes			Segregations regular for disjunction of 4th chromosomes	
	Type I Segregation (XY3/XT4)	Type II Segregation (X3/XYT4)		Type III Segregation (XYT/X34)	Type IV Segregation (XT/XY34)
			Diplo-4		
Haplo-4	Class 1 (Y)	Class 1 (no Y)	Translocation	Class 1 (Y)	Class 1 (no Y)
Cross #2	39.9†	2.4†	Cross #2	(63)	34.5*
Cross #3	109	5	Cross #3	204	104
Cross #4	105	2	Cross #4	64	83
Triplo-4	Class 2 (no Y)	Class 2 (Y)	Diplo-4 (3;4)	Class 2 (no Y)	Class 2 (Y)
Cross #2	118*	(5)	Cross #2	88.1*	(37)
Cross #3	...	...	Cross #3	...	...
Cross #4	186	3	Cross #4	123	78
Ratio of Haplo-4 Type I:Type II			Ratio of Translocation Type III:Type IV		
Cross #2 17:1			Cross #2 1.8:1		
Cross #3 22:1			Cross #3 2.0:1		
Cross #4 53:1			Cross #4 .77:1		
Ratio of Triplo-4 Type I:Type II			Ratio of 3;4 Type III:Type IV		
Cross #2 24:1			Cross #2 2.4:1		
Cross #3 ...			Cross #3		
Cross #4 62:1			Cross #4 1.6:1		

For meaning of symbols, see Table 3.

Type D (XXY;T(3;4)86D/T(3;4)86D/ci^D or spa^{Cat}) which is the designation given to the female used in Cross No. 5, carries a homozygous translocation instead of the heterozygous translocation which is present in the Type A female. The regular progeny from Cross No. 5 are heterozygous for the translocation and are genotypically similar except for those differences which result from the assortment of an extra Y and an extra fourth chromosome, i.e., the presence or absence of a Y chromosome, a fourth chromosome or both chromosomes. All classes of progeny are identifiable for the free fourth chromosome is marked with ci^D or spa^{Cat} and the presence of the Y is evidenced by its suppression of the dominant-brown variegation. Since the female parent in Cross No. 5 is triplo-4, the progeny are either diplo-4 or triplo-4 and thus Y-4 segregation can be measured without the need for a viability correction for haplo-4 flies.

If, in the female used in Cross No. 5, the assortment of the sex chromosomes and the free fourth chromosome are independent of each other, suppressed (extra Y) and unsuppressed (no extra Y) progeny are expected to appear in equal numbers among the diplo-4 and triplo-4 groups of flies. The results from Cross No. 5 are given in Table 5.

The data from Cross No. 5 are arranged in Table 6 with respect to the types of segregations involved and the classes of flies which result from each segregation type. Type-I segregation is defined as that in which the extra Y chromosome and the fourth chromosome assort to opposite poles and Type-II segregation is defined as that in which the Y chromosome and the fourth chromosome assort to the same pole. The relative frequency of Type-I and Type-II segregation can be measured by comparing the number of diplo-4 flies from Type-I with those from Type-II or by comparing the number of triplo-4 flies from Type-I with those from Type-II. The frequency of Type-I:Type-II segregation based on diplo-4 flies is 10:1 and the frequency based on triplo-4 flies is 13:1. The frequency with which the Y

TABLE 5

Results from Cross No. 5. Progeny of Type D females (y^2 v/y^2 v; Y^{C-S}; bw/bw; *T(3;4)86D*, bx^{34e} e^4/*T(3;4)86D*, bx^{34e} e^4/ci^D or spa^{Cat}) *and* v; In(2)bw^{VDe1}, bw^{VDe1}/SM1, al^2 Cy sp^2 *males*

Type of female parent	Regular disjunction of X chromosomes: Suppressed ♀ Triplo-4	Suppressed ♀ Diplo-4	Suppressed ♂ Triplo-4	Suppressed ♂ Diplo-4	Nonsuppressed ♀ Triplo-4	Nonsuppressed ♀ Diplo-4	Nonsuppressed ♂ Triplo-4	Nonsuppressed ♂ Diplo-4
ci^D	14	83	4	70	94	5	71	6
spa^{Cat}	10	148	8	148	150	18	164	15
Totals	24	231	12	218	244	23	235	21
	Secondary nondisjunction of X chromosomes							
ci^D	...	4	...	...	...	...	7	2
spa^{Cat}	...	6	...	...	...	...	5	3
Totals	...	10	...	...	...	...	12	5
	Percent regular for X's: 97.4				Percent nondisjuctional for X's: 2.6			

TABLE 6

Progeny of Type D females arranged with respect to the two types of segregation involved and the complementary classes which result from each type

	Type I Segregation (TY/T4)	Type II Segregation (T/TY4)
	Class 1 (Y)	Class 1 (no Y)
Diplo-4	449	44
	Class 2 (no Y)	Class 2 (Y)
Triplo-4	479	36
	Ratio Diplo-4 Type 1:Type 2 10:1	
	Ratio Triplo-4 Type 1:Type 2 13:1	

chromosome and the fourth chromosome are recovered together (Type-II progeny/Total progeny) is 7.9 percent whereas the expected frequency is 50 percent.

Only progeny which are products of the regular disjunction of the X chromosomes have been considered in the above results. Progeny which arise from nondisjunction of the X's comprise less than 4 percent of the total flies from each cross and their numbers are too small to analyze in detail.

A comparison of the total amount of nondisjunction of the X chromosomes in the four kinds of females under consideration presents a consistent pattern. The presence of a heterozygous translocation (Type B female) increases the amount of primary nondisjunction of the X's from .05 percent (BEADLE and STURTEVANT 1935) to .4 percent for T(3;4)86D and .4 percent for T(3;4)88B (Tables 1 and 2). The addition of a Y chromosome to a female which carries two normal X's is known to produce about 4.3 percent secondary nondisjunctional progeny (BEADLE and STURTEVANT 1935) or to have about eleven times the effect of these heterozygous translocations. The simultaneous presence of a Y and a heterozygous translocation (Type A female) does not have an additive effect but instead decreases the percentage of secondary nondisjunctional progeny to 1.7 percent for T(3;4)86D and 1.7 percent for T(3;4)88B (Tables 1 and 2). It is reasonable to expect that two Y chromosomes (which presumably form a bivalent), and a heterozygous translocation will reduce the percent of progeny nondisjunctional for the X chromosomes more than a homozygous translocation in the presence of one Y. This is borne out by the experimental results. When two Y chromosomes and heterozygous T(3;4)86D are present (Type C female), 1.4 percent secondary nondisjunctional progeny are observed (Table 1) whereas the presence of homozygous T(3;4)86D and one Y chromosome (Type D female) results in 2.6 percent secondary nondisjunctional progeny (Table 5).

DISCUSSION

Although it has been postulated that regions of homology may exist in the basal heterochromatin among all the chromosomes of *Drosophila melanogaster*

(GERSHENSON 1940; MULLER and PAINTER 1932; PROKOFYEVA-BELGOVSKAYA 1935) and although the possibility of associations formed in such regions between non-homologous chromosomes has been suggested as the mechanism responsible for certain enigmatic genetic data (COOPER, ZIMMERING and KRIVSHENKO 1955; SANDLER and NOVITSKI 1956), direct evidence in support of such associations is rather sparse.

Ideally, such evidence should be of two kinds. It should consist of observed cytological pairing between non-homologous chromosomes at the first meiotic metaphase and of genetic data clearly indicating the subsequent segregation of the paired non-homologues to different gametes in a significant frequency.

As the cytology of the meiotic events, particularly in the female of *Drosophila melanogaster* continues to be fairly refractory to analysis, no direct cytological evidence is available. Direct genetic evidence has been limited to segregations involving the sex and fourth chromosomes since zygotes aneuploid for entire autosomes other than chromosome four are lethal. Several types of gametes aneuploid for the sex and fourth chromosomes are recoverable as aneuploid zygotes, but under normal conditions pairing between homologues is so complete that there is no opportunity to observe whether the sex and fourth chromosomes possess the potentiality for non-homologous association and the consequent formation of these aneuploid types. It has been possible, however, through the use of unbalanced sets of chromosomes or structurally abnormal chromosomes to observe segregations which strongly suggest that such associations occur.

SANDLER and NOVITSKI (1956) have shown that the observed percentage of primary nondisjunction of X's is increased from .21 percent in sv^n diplo-4 flies to .68 percent in sv^n triplo-4 flies. Presumably, the extra four has interfered with the normal pairing of the X's by forming its own association with an X to cause the increase.

Similarly, LINDSLEY and SANDLER (1956) have reported the effect of an X duplication on the disjunction of the fourth chromosomes. Among 4524 progeny from attached-X females which also carry an X duplication, 29 haplo-4 flies were observed and 27 of these, or .59 percent, also carry the duplication. The non-homologous association in this case appears to be between the X duplication and a fourth chromosome and the amount of non-homologous pairing is related to the amount of nondisjunction of the fours.

In the two instances cited above one chromosome or a part of a chromosome has been added to the normal diploid complement. In each case, a potential univalent is now present and may interfere with the disjunction of a normal diploid pair. The frequency of nondisjunction of this pair is a measure of the degree of interference exerted by the extra chromosome or fragment.

It is only a step from this situation to one in which two extra chromosomal elements are present. Thus, GERSHENSON (1940) introduced three different duplications into triplo-4 males. Two of the three duplications did not assort randomly with the extra fourth chromosome but instead were recovered with a highly significant frequency in the diplo-4 progeny. The two duplications were of X-ray

origin, and although they carried the left tip of X, the remainder of the microchromosome was heterochromatic. The fragments were not distinguishable from the fourth chromosome on the metaphase plate. GERSHENSON points out that among 20 fragments that he had available, the three that were tested were selected because they had no significant effect on the disjunction of the X and Y in the male. Their effect on the disjunction of the X's in the female falls into GERSHENSON's minimal group and this effect does not appear to be significantly higher than that found by SANDLER and NOVITSKI to be caused by an extra fourth chromosome. It may well be that the fragments were in fact selected because they possessed a fourth chromosome centromere instead of an X chromosome centromere.

The present experiments make use of a much more favorable set of conditions for the study of this phenomenon, namely, an extra sex chromosome in the form of a Y and a 3–4 translocation. The translocation is expected to reduce pairing between the fourth chromosomes. For the first set of experiments, Type A females were developed to include a Y chromosome and a heterozygous 3–4 translocation. The two non-homologues (the Y and the normal fourth chromosome) go to opposite poles with a frequency of 78–83 percent instead of 50 percent as expected. The Y and the translocation go to opposite poles with a frequency of 62–68 percent instead of 50 percent. In other words, under these conditions, the Y chromosome and the fourth chromosome show a strong association. This association is stronger between the Y and the normal four than between the Y and the fourth chromosome which is involved in the translocation.

The situation in the second set of experiments (Type D females) is still more advantageous for the detection of nonrandom segregation of the Y and the fourth chromosomes. Type D females carry a Y, a free four and a homozygous 3–4 translocation instead of the heterozygous 3–4 translocation present in Type A females. BROWN (1940) found that in homozygous 3–4 translocations similar to T(3;4)-86D, crossing over and disjunction are normal. If the pairing requirements of the translocation are satisfied in the homozygous condition, the translocation would not be expected to become involved with the Y and segregate from it as it appears to do in the heterozygous condition. An increased frequency of Y-4 pairing and segregation to opposite poles might be expected. The results show that the Y and the fourth chromosome are recovered apart with a frequency of 92 percent in the progeny of Type D females (T(3;4)86D/T(3;4)86D/ci^D) in Table 5 as compared to a frequency of 85 percent in the progeny of Type A females (T(3;4)86-D/ci^D) in Table 3 if uncorrected for haplo-4 viability reduction. In other words, nondisjunction of the Y and the fourth chromosome occurs eight percent of the time when the mother is Type D and 15 percent of the time when the mother is Type A. This is a highly significant difference. When corrected for viability difference the corresponding percentages are eight percent and 16 percent.

If all pairing and subsequent disjunction, including heterochromatic pairing, implies homology, then the results obtained in these experiments may be taken to indicate that homologous regions are present in the Y and fourth chromosomes of

Drosophila melanogaster. On the other hand, since heterochromatin is often characterized by its ability to pair unspecifically with other heterochromatin, the observed Y-4 segregations may result from the general pairing affinity of all heterochromatin.

SUMMARY

1. The segregation of the sex chromosomes, the translocation and the free fourth chromosome has been simultaneously followed in females of *Drosophila melanogaster* which carry a normal Canton-S Y chromosome and which are heterozygous for a 3–4 translocation. A marked non random assortment between the Y and the free four and between the Y and the translocation has been observed for the two types of translocations which were used. When the translocation is one in which disjunction between the translocation and the free four is nearly random (T(3;4)86D), the Y and the free fourth chromosome are found to segregate to opposite poles with a frequency of 83 percent; the Y and the translocation segregate to opposite poles with a frequency of 62 percent. When the translocation is one in which disjunction between the translocation and the free fourth chromosome is more regular (T(3;4)88B), the Y and the free fourth chromosome are found to segregate to opposite poles with a frequency of 78 percent; the Y and the translocation segregate to opposite poles with a frequency of 68 percent.

2. The segregation of the sex chromosomes, the translocation and the free fourth chromosome has been followed in triplo-4 females which carry a normal Canton-S Y chromosome and which are homozygous for a 3–4 translocation (T(3;4)86D). The non random assortment of the Y and the free four is more pronounced in this case. The Y and the fourth chromosomes are found to segregate to opposite poles with a frequency of 92 percent as compared to a frequency of 83 percent when the same translocation is heterozygous.

3. The observed non random assortment between the Y chromosome and the free fourth chromosome or between the Y chromosome and the translocation is interpreted as arising from the association between and subsequent disjunction of the Y and the fourth chromosome or the translocation. Whether the postulated association implies homology remains open to question.

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ACTION OF MANGANOUS CHLORIDE ON INDUCED SOMATIC SEGREGATION IN PENICILLIUM CHRYSOGENUM DIPLOIDS

G. SERMONTI AND G. MORPURGO

Istituto Superiore di Sanità, International Research Centre for Chemical Microbiology, Rome, Italy

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NITROGEN mustard (HN–2), at doses which bring about almost total inactivation of the treated conidia, has a powerful stimulating effect on somatic segregation in some heterozygous *Penicillium chrysogenum* diploids (MORPURGO and SERMONTI 1959). On plating the surviving conidia, color marker segregants appear as yellow or white entire colonies, or yellow or white sectors emerging from the green phenotype of the heterozygous diploid colonies. The color of the colonies and sectors is due to the color of the conidia which they produce. Some colonies with colored sectors obtained after HN–2 treatment give rise to clones whose conidia produce colonies almost all of which show sectors segregant for color. Each one of these "unstable" clones displays its own individual segregation pattern (MORPURGO and SERMONTI 1959). Similar unstable clones have been obtained from X-ray irradiated conidia.

The inactivation produced by HN–2 is strongly reversed by the action of manganous chloride ($MnCl_2$) added to the plating medium; however, this salt has no reactivating effect on conidia inactivated by X-ray irradiation (SERMONTI and MORPURGO 1958). The object of the present research was to establish the effect of $MnCl_2$ on the somatic segregation processes induced directly or indirectly by HN–2. Some further experiments concern the same problem in connection with X-ray-induced segregation.

MATERIALS AND METHODS

Strains: The strains used have already been described in MORPURGO and SERMONTI (1959), of which the present paper is a continuation. They are all descended from heterozygous diploid XXXIV of *Penicillium chrysogenum*, of which the phenotype is green and the genotype may be symbolized as follows:

$$\frac{Y\ PY\ w\ cy}{y\ py\ W\ CY}$$

The strains used were:

Strain XXXIV S, pyridoxineless, diploid, of genotype *Y py w cy/y py W CY*;

Unstable strain B, whose conidia produce green colonies with extensive yellow pyridoxineless and/or white cysteineless sectors;

Unstable strain D, whose conidia produce green prototrophic colonies with extensive yellow prototrophic sectors;

Unstable strain F, with colonies of similar phenotype to strain D;

Unstable strain N, with green colonies producing white cysteineless sectors later.

All these strains were obtained from conidia which had survived HN–2 treatment. Unstable strain 3X was obtained from a colony of strain XXXIV S which had survived X-ray irradiation. It produces yellow pyridoxineless sectors and stable dark-green pyridoxineless sectors.

Media: The complete medium described in the earlier work was used in the present tests.

Use of nitrogen mustard: Methyl-bis(β-chloroethyl)amine (HN–2) was used in the manner described in the previous paper. Treatment was interrupted after one minute or after four minutes, giving 95 percent and 99.9 percent inactivation respectively of the treated conidia.

Use of X-rays: X-ray irradiation was carried out on conidia in aqueous suspension in glass test tubes. A 245KV X-ray tube was used at 15 mA, filtration 1 mm Al, exposure 50,000r.

Use of manganous chloride: $MnCl_2.4\ H_2O$ was added to melted complete agar at a concentration of 0.8 percent (w/v), unless otherwise stated. It corresponds to 40 mM.

RESULTS

Effect of $MnCl_2$ on induced somatic segregation

Immediate segregation: This takes the form of the appearance of some completely yellow or white colonies directly from conidia which have survived HN–2 treatment or X-ray irradiation. The white colonies have been disregarded in this part of the work because their phenotype is easily confused with that of the conidialess colonies which sometimes appear after treatment with mutagenic agents.

After HN–2 treatment the conidia of heterozygous diploid XXXIV S were plated on Petri dishes containing complete medium with and without the addition of $MnCl_2$. Since conidia inactivated by HN–2 are strongly reactivated (SERMONTI and MORPURGO 1958) in the presence of $MnCl_2$, the conidium suspensions were suitably diluted before plating on the agar containing the salt.

Untreated conidia gave rise exclusively to green colonies, with no yellow or otherwise colored colonies either in the presence or in the absence of $MnCl_2$. Conidia treated with HN–2 gave rise to a high proportion of yellow colonies on unsupplemented medium and a proportion 3–5 times lower of yellow colonies on medium supplemented with $MnCl_2$ (Table 1).

Similar experiments were performed with conidia which had survived X-ray irradiation (50,000r). They were plated at the same density in the presence and in the absence of $MnCl_2$, since the salt has no reactivating effect on irradiated conidia (SERMONTI and MORPURGO 1958). The proportion of yellow colonies is

TABLE 1

Effect of $MnCl_2$ on induced segregation of the marker "yellow" from diploid XXXIV S

Mutagenic agent	Exposure	Supplement to the plating medium	Surviving conidia (Percent)	Colonies observed (No.)	Yellow colonies (No.)	Yellow colonies as percentage of total observed (Percent)
....	....	.....	100	107	0	0
....	....	$MnCl_2$ (40 mM)	106	114	0	0
HN–2	1 min	.....	4.7	813	51	6.31±0.85
HN–2	1 min	$MnCl_2$ (40 mM)	36.7	1,098	20	1.82±0.40
HN–2	4 min	.....	0.09	103	12	11.66±3.16
HN–2	4 min	$MnCl_2$ (40 mM)	14.0	601	14	2.33±0.62
X-rays	50,000r	.....	0.42	269	23	8.55±1.70
X-rays	50,000r	$MnCl_2$ (40 mM)	0.22	143	3	2.09 – 1.43

strongly reduced in the presence of $MnCl_2$ (Table 1, last two lines). $MnCl_2$ therefore acts as a corrective of chromosomal aberrations induced by X-rays although this effect is not accompanied by any restoration of vitality such as was observed in HN–2 treated conidia.

Reconstruction experiments have been carried out to check whether $MnCl_2$ had any selective effect against yellow segregants induced by HN–2. Conidia were harvested from a number of yellow segregant colonies and from an equal number of green nonsegregant colonies surviving HN–2 treatment. The mixed suspension was plated on agar supplemented or unsupplemented by $MnCl_2$. As shown in Table 2 a feeble selective effect against the yellow segregants was

TABLE 2

Reduction by $MnCl_2$ of the rate of yellow induced segregants from diploid XXXIV S, as compared with the effect of $MnCl_2$ in reconstruction experiments

Media	Exp.	Colonies from plating of green conidia treated with HN–2			Colonies from plating of yellow and green conidia*			χ^{2}‡
		Green (No.)	Yellow (No.)	Ratio Yellow/Green (Corrected)†	Green (No.)	Yellow (No.)	Ratio Yellow/Green (Corrected)†	
Complete (C)	1	595	43	(1)	342	260	(1)	....
Complete (C)	2	272	28	(1)	111	104	(1)	....
C+$MnCl_2$ (20 mM)	2	538	29	0.497	138	112	0.867	13.16
C+$MnCl_2$ (30 mM)	2	612	18	0.286	105	67	0.734	18.77
C+$MnCl_2$ (40 mM)	1	1,798	34	0.261	324	169	0.687	42.71

* Harvested at random from about 20 yellow and 20 green colonies grown on complete medium from HN–2 treated conidia.
† Ratios expressed relative to those on C medium, in corresponding experiment, taken as unity.
‡ Comparison of the corrected ratios: yellow/green, on the same medium. (d.f. = 1).

actually observed. This has been confirmed by the observation that induced yellow segregants grown on $MnCl_2$-agar display a significant selective advantage over the green nonsegregant colonies when their conidia are tested in reconstruction experiments as above. The selective effect explains only partially the reduction of the segregation by $MnCl_2$. A significant specific effect on the segregation rate must be invoked to explain the residual diminution.

Action of other bivalent-cation salts on the induced somatic segregation: $MgCl_2$ and $CaCl_2$ have been tested, in the same condition as $MnCl_2$, at the following doses: 50 mM, 100 mM and 200 mM, and $ZnCl_2$, which is more toxic to Penicillium, at doses of 3 mM, 6 mM and 10 mM. The test strain was XXXIV S treated with HN–2, with a survival rate of 0.03 in the absence of the salts. No significant reduction in the frequency of the yellow segregants was observed with any of the tested salts. $MnCl_2$, at the doses of 30 mM and 50 mM, gave a threefold reduction of yellow segregants in the same experiment.

Effect of $MnCl_2$ on spontaneous segregation from unstable clones: Conidia of four different unstable clones set up after HN–2 treatment were plated on complete medium in the presence and in the absence of $MnCl_2$. The proportion of colonies with and without sectors which had grown on the dishes was observed after at least ten days' growth. The general picture is the same for all the strains (Table 3): in the absence of $MnCl_2$ almost all the colonies show the characteristic mosaic pattern (Figure 1) while in the presence of $MnCl_2$ almost all the colonies are green, with no trace of sector formation (Figure 2), even when the colonies have reached the size associated, in colonies grown in the absence of the salt, with the regular appearance of colored segregant sectors.

In some experiments the conidia plated consisted of a mixture of green conidia from an unstable strain and conidia obtained from color segregant sectors of the same strain. The relative sizes of colonies derived from green and segregant conidia were substantially the same in the absence (Figure 3) and in the presence (Figure 4) of $MnCl_2$ for each of the strains observed. Furthermore, the relative frequency of occurrence of the two types of colonies shows no great difference on the two media, although perhaps a greater relative rate of survival of segregant colonies may be observed on the medium with $MnCl_2$ than on the unsupplemented medium (Table 4). These observations make it very improbable that the effect

TABLE 3

Effect of $MnCl_2$ on spontaneous segregation of the markers "yellow" and "white" from unstable strains

Strain	Color of sectors	Supplement to the plating medium	Colonies observed		
			Mosaic colonies (No.)	Without sectors (No.)	Unclassified (No.)
B	Yellow and white	.....	288	24	22
		$MnCl_2$ (20 mM)	78	251	16
D	Yellow	.....	262	0	0
		$MnCl_2$ (40 mM)	0	97	0
F	Yellow	.....	125	7	1
		$MnCl_2$ (40 mM)	6	250	0
3X	Yellow and dark green	.....	71	0	59
		$MnCl_2$ (40 mM)	0	38	7

of $MnCl_2$ in suppressing the spontaneous segregation of unstable clones may be explained by the occurrence of a selective effect against the segregants.

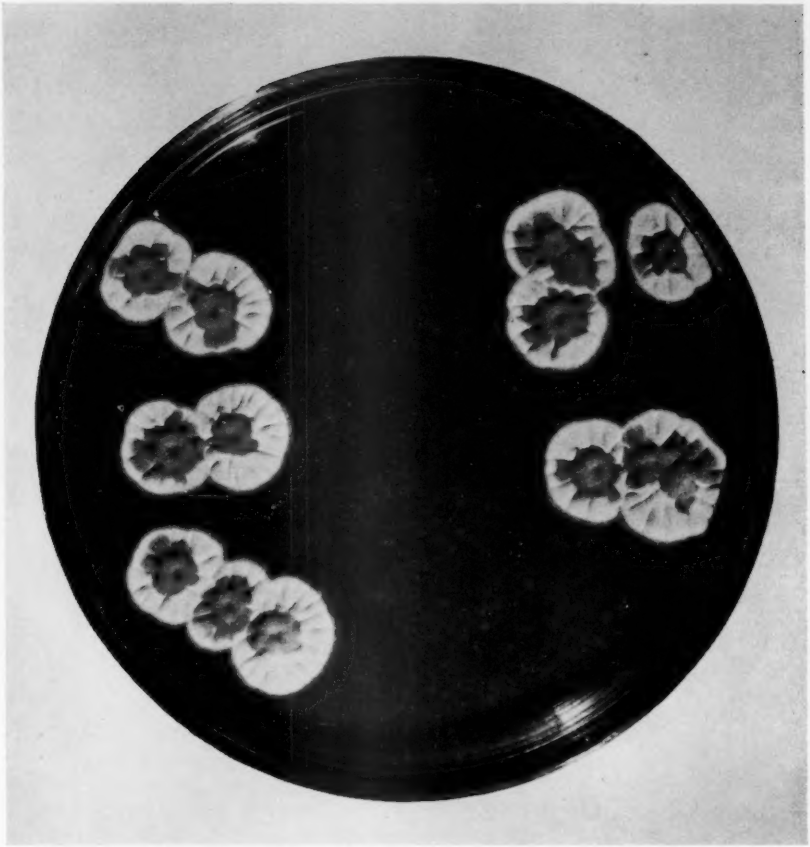

FIGURE 1.—Colonies of unstable strain D grown on complete medium showing wide yellow sectors.

The effect of $MnCl_2$ in inhibiting spontaneous segregation was also observed with one clone whose instability had been set up by X-ray irradiation, clone 3X (Table 3, last two lines).

Action of other bivalent-cation salts on the spontaneous segregation of unstable clones: A series of experiments was carried out in which different bivalent-cation salts were added to the complete medium in the hope of observing some effect similar to that of $MnCl_2$. The test strains used were strain D and in some cases strain B. All the salts were used at doses sufficient to limit growth in the colonies tested, except in the case of calcium and magnesium salts, which were used in concentrations equimolecular with the 0.8 percent $MnCl_2$ solution (40mM). The following salts were tested: $(CH_3COO)_2Cu$, $ZnCl_2$, $FeSO_4$, $NiSO_4$, $CoSO_4$,

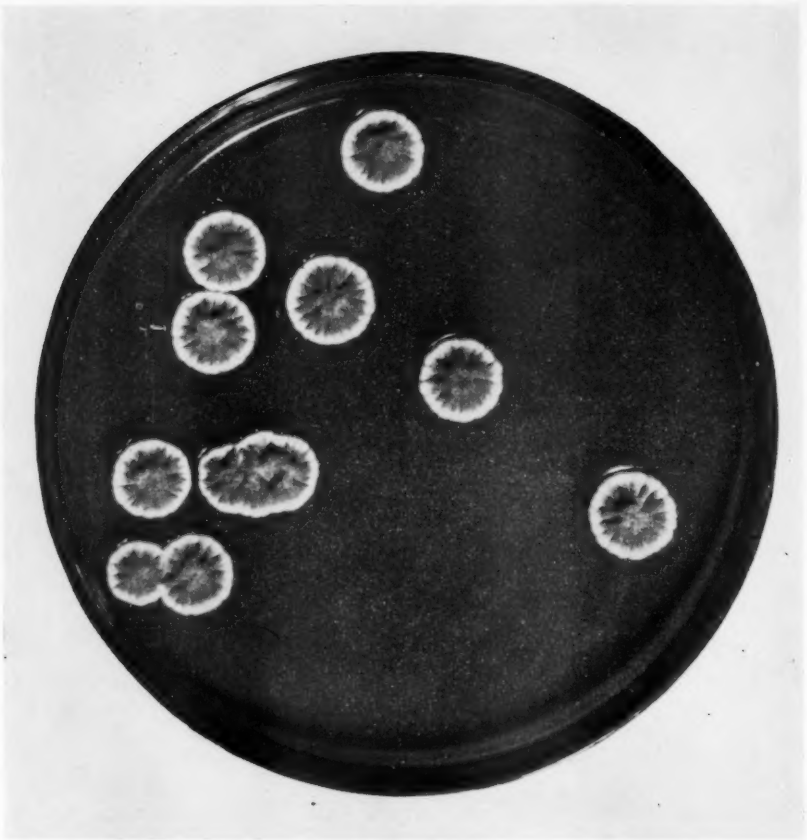

FIGURE 2.—Colonies as in Figure 1 on complete medium supplemented with $MnCl_2$ (40 mM). No sectors are produced.

$CaSO_4$, and $MgSO_4$. None of these produced any reduction in the rate of occurrence of mosaic colonies in the unstable strains, although at the doses used some of them had a marked effect on the size, morphology, and color of the treated colonies.

Progeny of unstable clones grown in the presence of $MnCl_2$: Conidia were collected from six colonies grown in the presence of $MnCl_2$ belonging to unstable strain D (yellow sectors) and seeded separately in the absence of the salt at a density of about 30 conidia per dish. The same operation was carried out on six colonies of the same strain grown in the absence of $MnCl_2$. In the latter case the conidia were taken from the green central zone, avoiding the sectors, while in the

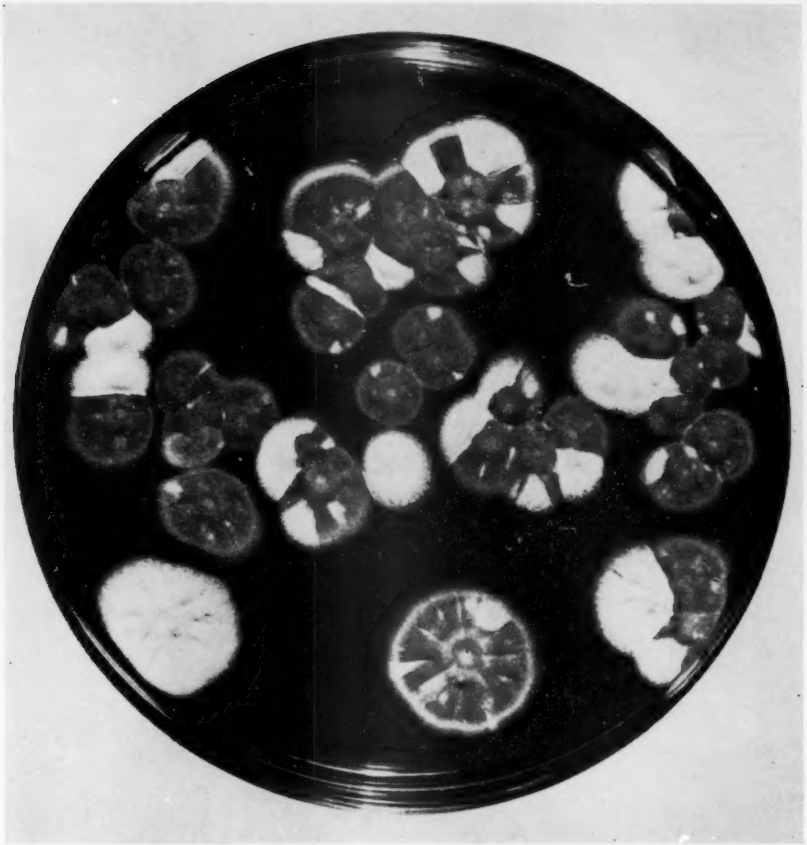

FIGURE 3.—Colonies of unstable strain N grown on complete medium, showing white sectors. The white colonies derive from plating of segregant conidia.

first case they were taken from over the whole spore-bearing surface, there being no sectors at all. All twelve populations of conidia obtained in this way produced 100 percent of characteristic mosaic colonies, independently of the origin of the conidia used for plating. It is worth mentioning that not a single completely yellow colony grew from hundreds of conidia taken from colonies grown in the presence of $MnCl_2$; this suggests that segregation is completely inhibited by $MnCl_2$. However, this suppression is reversible.

An exactly analogous result was obtained on plating conidia from two colonies of unstable strain N (white sectors) grown in the presence of $MnCl_2$. A parallel plating was carried out from the green part of two colonies grown on a medium

FIGURE 4.—Colonies as in Figure 3 on complete medium supplemented with $MnCl_2$ (40 mM). No sectors are produced. The relative size of the unstable and white colonies are the same as in Figure 3.

unsupplemented with $MnCl_2$. The appearance of the platings obtained from the two types of seeding was exactly analogous. With this strain again the conidia derived from colonies grown in the presence of $MnCl_2$ never gave rise to completely white colonies.

DISCUSSION

Alongside the reviving effect of $MnCl_2$ on conidia treated with nitrogen mustard (HN–2) (SERMONTI and MORPURGO 1958), a second marked effect of this salt has appeared in the course of the present work. $MnCl_2$ added to the plating

TABLE 4

Effect of $MnCl_2$ on survival in unstable clones and their spontaneous segregants, after mixed plating

Strain	Color of segregants	Supplement to the plating medium	Total numbers of colonies observed: Green (unstable) (No.)	Yellow (segregants) (No.)	Ratio Yellow/Green	χ^2 (d.f.=2)
		.....	37	117	3.16	
D	Yellow	$MnCl_2$(20 mM)	31	143	4.61	8.16
		$MnCl_2$(40 mM)	18	123	6.83	
		.....	127	39	0.30	
F	Yellow	$MnCl_2$(20 mM)	54	23	0.43	5.29
		$MnCl_2$(40 mM)	71	40	0.56	
		.....	284	68	0.24	
N	White	$MnCl_2$(20 mM)	104	39	0.37	7.96
		$MnCl_2$(40 mM)	256	99	0.39	

medium strongly reduces the rate of occurrence of the chromosome rearrangements induced both by HN–2 and by X-ray irradiation; this is deduced from the rate of occurrence of phenotypes showing recessive markers of the heterozygous diploid treated. The antisegregant effect of $MnCl_2$ appears in two different types of experiment. On the one hand $MnCl_2$ partially neutralizes the direct effects of HN–2 and X-ray irradiation; this appears when the treated conidia are plated in the presence of adequate concentrations of the salt. On the other hand, it almost completely (although reversibly) neutralizes the indirect effect of these mutagenic agents, *viz.* the chromosome rearrangements which appear as a consequence of a hereditary condition of nuclear instability set up by the mutagenic treatment and preserved indefinitely. This effect is only observable when conidia of unstable strains are plated in the presence of $MnCl_2$. It takes the form of an almost complete absence of mosaic colonies among the colonies grown from these conidia.

Nothing can be deduced at the moment from the present authors' work concerning the mechanism of action of $MnCl_2$, but an interpretation is strongly suggested by the experiments of ERNSTER *et al.* on the action of Mn^{++} on mitochondrial organization. According to these authors (ERNSTER 1956) the change of mitochondrial organization elicited by factors which induce a release of mitochondrial ATP may take the form of a disconnection of the coupling of phosphorylation to electron transfer. This effect can be reversed by supplementing the uncoupled system with suitable levels of ATP and Mn^{++} (LINDBERG and ERNSTER 1954).

Uncoupling of oxidative phosphorylation has been observed in animal tissues after X-ray irradiation (VAN BEKKUM 1956) and, granted the close analogy between the actions of HN–2 and X-rays, it may be postulated that it also takes place after HN–2 treatment. Oxidative phosphorylation is the principal source of ATP in aerobic organisms.

WOLFF and LUIPPOLD (1956) consider, on the basis of a number of observations, that "the chemical bonds formed in rejoining of radiation induced chromosome breaks require ATP as a source of energy for their synthesis". Dinitrophenol inhibition of ATP formation inhibits the rejoining of chromosome breaks (WOLFF and LUIPPOLD 1955) while the addition of exogenic ATP accelerates the rejoining process (WOLFF and LUIPPOLD 1956).

The simplest explanation of the phenomena described in this paper on the basis of the results quoted is as follows. In the case of the immediate effects of the mutagenic agents used and their cancellation by $MnCl_2$, it is reasonable to suppose that HN–2 and X-ray irradiation cause chromosome breaks with consequent loss of the acentric fragments. These phenomena would be observable in heterozygous diploid Penicillium as the appearance of segregants showing the recessive markers lying on the segment of chromosome corresponding to the lost segment.

The action of the mutagenic agents would also bring about a change in the system which controls oxidative phosphorylation, *viz.* ATP synthesis. Two independent effects of X-ray irradiation, one direct effect on chromosome breaks and another on the "rejoining system", have already been postulated (WOLFF and ATWOOD 1954). ATP deficiency would delay the rejoining of the chromosome breaks and probably reduce its efficiency. Restoration of oxidative phosphorylation by means of $MnCl_2$ could bring ATP production back towards normal and thus reduce the proportion of unrepaired chromosome breaks.

The effect of $MnCl_2$ on the spontaneous segregation of unstable strains is more difficult to interpret, on account of the problematic nature of the phenomenon itself (MORPURGO and SERMONTI 1959).

SUMMARY

Nitrogen mustard (HN–2) produces a sharp increase in somatic segregation in a heterozygous diploid of *Penicillium chrysogenum*. This takes the form of the appearance of colonies displaying recessive markers of the diploid treated. HN–2 also sets up unstable clones showing a high rate of occurrence of spontaneous segregation for particular markers (MORPURGO and SERMONTI 1959). The addition of manganous chloride ($MnCl_2$) to the plating medium of conidia treated with HN–2 appreciably lowered the rate of appearance of the segregants induced by HN–2.

Similar phenomena were observed when X-ray irradiation was used as the stimulus of somatic segregation.

$MnCl_2$ is known to be a powerful reactivating agent for conidia inactivated by HN–2, although it is without effect on conidia inactivated by X-ray irradiation (SERMONTI and MORPURGO 1958).

Unstable clones grown on medium supplemented with $MnCl_2$ practically ceased to produce segregant sectors. This effect was reversible: plating of conidia of colonies of unstable clones grown in the presence of $MnCl_2$ on an unsupplemented medium gave rise once again to colonies displaying the instability characteristic of the clone.

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TRANSDUCTION TO PENICILLIN AND CHLORAMPHENICOL RESISTANCE IN SALMONELLA TYPHIMURIUM

S. BANIČ[1, 2]

Carnegie Institution of Washington, Department of Genetics, Cold Spring Harbor, New York

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TO explain multistep resistance to antibiotics, DEMEREC (1945, 1948) assumed that there are a number of genes that affect bacterial resistance. If one of them mutates, the bacterial cell acquires resistance to a certain concentration of the antibiotic. The combined effect of mutations in several genes is a high degree of resistance or complete resistance. In penicillin type of resistance the different genes affecting resistance are according to DEMEREC about equally potent, so that the resistance of bacterial individuals in which only one gene has mutated is never very high. In the streptomycin type of resistance, however, the genes for resistance vary greatly in potency: a mutation in a less potent gene will be responsible for a low degree of resistance, a mutation in a highly potent gene for a high degree of resistance.

CAVALLI and MACCACARO (1952) studied the genetic mechanisms of chloramphenicol resistance in *E. coli* K12 by the recombination method. The results were in good agreement with the polygenic theory. The alternative possibility of a system of multiple alleles entailing various degrees of resistance was excluded on the basis that (a) the resistance genes, when crossed together, give rise to a certain proportion of sensitives, and (b) if crossed to marked sensitives, they show different linkages with the markers.

Later DEMEREC *et al.* (1955) obtained evidence of complex loci in *Salmonella typhimurium* by means of the transduction technique. They made tests to determine allelic relations among 25 cystine-requiring mutants and found that transduction to wild type occurred in all but a very few combinations. They concluded that mutational changes occurring at different sites within a gene locus, which extends over a section of chromosome, give rise to nonidentical alleles.

With these findings in mind, I decided to study the genetic mechanisms of multistep resistance by means of the transduction technique. It appeared that theoretically two mechanisms might be expected: a polygenic system; and, in addition, mutations at different sites of the same gene locus.

MATERIALS AND METHODS

Media and drugs: In the experiments to be described below nutrient broth and nutrient agar, soft agar, penicillin G, and chloramphenicol were used.

[1] Fellow of the Yugoslav Foundation, Sklad Borisa Kidriča.

[2] Present address: Institute of Microbiology, Medical Faculty, Ljubljana, Yugoslavia.

Strains: The bacteria used were *Salmonella typhimurium* strain LT-2; a prolineless mutant *proD-129;* three first-step penicillin-resistant mutants; one each second-, third-, and fourth-step penicillin-resistant mutants; and one each first-, second-, and third-step chloramphenicol-resistant mutants. The transducing phage was PLT–22.

Designation of strains: Penicillin-resistant mutants are designated by the abbreviation *pen-r* and chloramphenicol-resistant mutants by *chl-r*. A number attached to the symbol *pen-r* or *chl-r* (for example, *pen-r-1*) identifies the strain. Multistep resistant mutants are identified by two or more numbers (for example, *pen-r-1-2*, a second-step resistant mutant isolated from *pen-r-1*).

Methods: In the first attempts to transduce first-step resistance to penicillin and chloramphenicol, a double-agar-layer technique was employed. The results were encouraging but not satisfactory. An aerated-broth-culture method was therefore worked out. Adsorption took place in a bubbler tube containing 0.5 ml of an overnight broth culture of the recipient strain (about 2×10^9 bacteria per ml) mixed with 0.5 ml of a phage preparation from the donor strain. After six minutes of incubation in a water bath at 37°C, nine ml of broth were added and the tube was aerated in the water bath at 37°C until a density of approximately 2×10^9 bacteria per ml was reached, in order to allow for phenotypic expression of the resistant transductants. When the recipient strain was LT–2 or a relatively fast-growing mutant, this density was obtained in 3–4 hours; when the recipient was *proD-129*, which is slow growing, six or more hours of incubation were necessary. A control tube in which the bacteria were mixed with a homologous phage was treated in the same way as the transduction tube.

An appropriate quantity of the antibiotic was pipetted into one side of an empty Petri dish. An 0.1 ml sample of the culture was taken from the tube and pipetted into the opposite side of the Petri dish. Then 20 ml of melted nutrient agar, adjusted to 45°C, was poured into the Petri dish. The bacteria and antibiotic were well mixed with the agar by moving the plate in different directions for one minute. Three plates each were prepared from the transduction tube and the control tube. The density of the broth cultures in the transduction and control tubes was then checked with a Klett-Summerson apparatus to be sure that there was no significant difference in density between the transduction and control cultures. The plates were kept in the incubator at 37°C for three days, after which the colonies were counted on both series of plates.

Most of the experiments were done in this manner; but eventually the double-agar-layer technique was tried again, because it appeared that at least in some instances that method might be superior. It was realized that the prerequisite for successful transduction of first-step resistance by that technique is a completely even table, since otherwise a gradient plate is obtained that makes an exact colony count impossible. Furthermore, a new detail was introduced into the technique, which proved to be important. After adsorption was carried out in the usual way, four ml of broth were added before plating. This was done, first, because it was desirable to reduce the number of colonies developing from spontaneous mutants

and, second, because bacterial growth in agar is inhibited if the number of bacteria is too great (EAGLE *et al.* 1955). The further procedure was as follows. Samples of 0.1 ml were taken from the cultures and pipetted onto the bottoms of empty plates. Then 20 ml of melted agar were poured into each plate and mixed well with the culture, as described above. After hardening of the agar the plates were incubated at 37°C for three hours. They were then removed from the incubator and five ml of soft agar containing the desired quantity of antibiotic were poured into each plate on top of the first agar layer. The plates were held for two hours at room temperature to allow diffusion of the antibiotic, and then placed again in the incubator; after three days the colonies were counted.

RESULTS

Transduction to penicillin resistance: The aerated-broth-culture method proved to be satisfactory for the transduction of first-step penicillin resistance. The difference in numbers of colonies on the transduction and on the control plates was significant. Better results were obtained with the slow-growing strain *proD-129* as recipient than with strain LT–2. Resistant bacteria are as a rule more slow growing than the sensitive wild type, and the same is true of the transductants. When the faster growing wild type strain is employed as recipient, nontransduced sensitive cells have a selective advantage over the resistant transductants under the conditions of this broth technique. It seems probable that when the recipient strain is a slow-growing one, the difference in growth rate between sensitive and transduced resistant cells is less. The concentration of penicillin employed was 20 units; on this about 50 percent of plated bacteria of a first-step resistant mutant of strain LT–2 are able to survive.

TABLE 1

Numbers of penicillin-resistant colonies observed in transduction experiments LT–2 (×) pen-r-10 *and* proD-129 (×) pen-r-10 (*20 units penicillin per ml of agar*)

Recipient	Donor	Experiment 1		Experiment 2	
		Transd.	Control	Transd.	Control
LT–2	*pen-r-10*	376	97	157	42
proD-129	*pen-r-10*	406	37	501	43

The results of transduction of strains LT–2 and *proD-129*, respectively, by the first-step resistant donor *pen-r-10* are summarized in Table 1. With strain LT–2 as recipient, the transduction plates contained 3.8 times as many colonies as the control plates in one experiment, and 3.7 times as many in another experiment. With *proD-129* as recipient, the number of colonies on the transduction plates was larger by a factor of 10.9 in the first experiment and 11.9 in the second experiment.

The same technique was employed in transductions of the sensitive strain by second-, third-, and fourth-step resistant mutants. In these experiments two concentrations of penicillin were employed: 20 units, corresponding to the resistance

level of the first step, and 60 units, corresponding to that of the second step. The results showed that multistep resistant strains as donors are able to transfer only a degree of resistance corresponding to first-step resistance. The results of transduction on the plates containing 20 units of penicillin per ml of agar are presented in Table 2.

TABLE 2

Results of transduction experiments proD-129 (×) pen-r-1, pen-r-1-2, pen-r-1-2-3, *and* pen-r-1-2-3-4 (*20 units penicillin per ml of agar*)

Donor	Experiment 1		Experiment 2		Experiment 3	
	Transd.	Control	Transd.	Control	Transd.	Control
pen-r-1	69	11	396	63	310	32
pen-r-1-2	233	21	261	23	225	37
pen-r-1-2-3	71	17	64	23	101	27
pen-r-1-2-3-4	59	19	52	23	61	25

Experiments were next made to test whether first-step resistant strains could be transduced to second-step resistance (resistance to 60 units of penicillin) by other first-step resistant strains. Strain *pen-r-5* was found to be transducible to second-step resistance by strain *pen-r-1* or *pen-r-10*. The results are summarized in Table 3. Attempts to transduce strains *pen-r-1* and *pen-r-10* by strain *pen-r-5*

TABLE 3

Results of transduction experiments pen-r-5 (×) pen-r-10 *or* pen-r-1 (*60 units penicillin per ml of agar*)

Donor	Experiment 1		Experiment 2		Experiment 3		Experiment 4	
	Transd.	Control	Transd.	Control	Transd.	Control	Transd.	Control
pen-r-10	236	37	113	20	103	25	221	27
pen-r-1	251	40	126	32	149	29	164	26

failed, however, although such transduction was to be expected. This finding suggested that the broth technique is not sensitive enough for the demonstration of transduction in all cases.

New attempts were therefore made to work out a satisfactory double-agar-layer technique, which might be expected to be more sensitive than the broth technique in cases of low transduction frequency and in cases of large difference in growth rate between the sensitive recipient strain and resistant transductants. The improved technique described in the Methods section was employed. Results of transduction to first-step resistance, with *proD-129* as recipient and strains *pen-r-10* and *pen-r-1-2* as donors, were very satisfactory; they are presented in Table 4. With this technique it was also possible to transduce strain *pen-r-10* to

TABLE 4

Results of transduction experiments proD-129 (×) pen-r-10 *and* proD-129 (×) pen-r-1-2, *made with the double-agar-layer technique (20 units penicillin per ml of agar)*

Donor	Transd.	Control
pen-r-10	88	7
pen-r-1-2	58	5

the second step with strain *pen-r-5* as donor (Table 5). Thus this technique proved more sensitive than the broth method.

TABLE 5

Results of transduction experiments pen-r-10 (×) pen-r-5, *made with the double-agar-layer technique (60 units penicillin per ml of agar)*

Donor	Experiment 1		Experiment 2		Experiment 3	
	Transd.	Control	Transd.	Control	Transd.	Control
pen-r-5	35	4	36	3	30	11

Transduction to chloramphenicol resistance: The aerated-broth-culture method was used successfully in transduction to chloramphenicol resistance. In this case, too, strain *proD-129* proved to be a more appropriate recipient than strain LT–2. The experiments were restricted to transduction of strains *proD-129* and LT–2 by donor strains *chl-r-1*, *chl-r-1-2*, and *chl-r-1-2-3*. When the first-step resistant strain *chl-r-1* was the donor, only one concentration of chloramphenicol was used, that is, 8 μg. When second-step (*chl-r-1-2*) and third-step (*chl-r-1-2-3*) resistant strains were donors, two concentrations were employed, namely, 8 μg and 30 μg. On the plates containing 30 μg of chloramphenicol, no colonies developed. Thus with second- and third-step resistant strains as donors it was possible to transfer only a degree of resistance corresponding to first-step resistance. The data for the tranduction plates containing 8 μg of chloramphenicol are given in Tables 6 and 7.

TABLE 6

Numbers of chloramphenicol-resistant colonies observed in transduction experiments proD-129 (×) chl-r-1, chl-r-1-2, *and* chl-r-1-2-3 *(8 μg chloramphenicol per ml of agar)*

Donor	Experiment 1		Experiment 2		Experiment 3	
	Transd.	Control	Transd.	Control	Transd.	Control
chl-r-1	72	16	46	10	66	35
chl-r-1-2	527	19	287	11	248	30
chl-r-1-2-3	159	15	172	17	183	28

TABLE 7

Results of experiments LT–2 (×) chl-r-1, chl-r-1-2, *and* chl-r-1-2-3
(*8 μg chloramphenicol per ml of agar*)

Donor	Transd.	Control
chl-r-1	56	52
chl-r-1-2	64	5
chl-r-1-2-3	60	34

DISCUSSION

Of the two techniques employed for transduction to penicillin or chloramphenicol resistance, the double-agar-layer technique appears to be preferable, although most of the experiments in this study were done by the aerated-broth-culture method. In both procedures the concentration of antibiotic is very important. According to our experience, it is best to choose a concentration which allows about 50 percent survival of the corresponding resistance step. A lower concentration of the antibiotic increases the number of transductants but increases still more the number of spontaneous mutants, which is unfavorable for the experimental results.

In the present study I was interested primarily in the question of whether it is possible to transduce bacteria to first-step penicillin or chloramphenicol resistance. It might be expected *a priori* that there was very little chance of working out an appropriate technique for transduction to first-step resistance, because of the high spontaneous-mutation rate. Results obtained by the techniques described, however, show that it is possible to transfer first-step resistance by transduction. I was interested further in finding out whether it is possible to transfer the full resistance of second-, third-, or fourth-step strains to a sensitive recipient. The results show that it is not possible to produce more than first-step resistance by transduction with phage grown on multistep resistant strains. From this it can be inferred that different genes determining penicillin or chloramphenicol resistance in strain LT–2 are not closely linked. The results indicate that mutations at different sites of the same gene locus do not have a significant role in the building up of multistep resistance.

A third question of interest was whether it is possible to build up multistep resistance to penicillin by the transduction method. It was found that transduction of a first-step resistant strain by another independent first-step resistant mutant results in second-step resistance.

On the basis of our data the following conclusions may be drawn: (a) resistance to penicillin or chloramphenicol in *Salmonella typhimurium* is gene controlled, because it is possible to transfer it to a sensitive recipient by transduction techniques; (b) there is a polygenic system governing such resistance; (c) the different genes for resistance to penicillin or to chloramphenicol are not linked.

SUMMARY

An aerated-broth-culture method as well as a double-agar-layer technique were worked out by which it is possible to transduce sensitive strains of *Salmonella typhimurium* by first-step and multistep penicillin- or chloramphenicol-resistant mutants. Multistep resistant mutants are capable of transducing sensitive strains only to a degree of resistance corresponding to first-step resistance. This means that separate genes controlling resistance to penicillin or to chloramphenicol are not linked. Transduction of first-step penicillin-resistant strains by independent first-step mutants gives rise to second-step resistance. These data confirm the hypothesis that a polygenic system governs multistep resistance.

ACKNOWLEDGMENT

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CHROMOSOME FRAGMENTS PARTICIPATING IN TRANSDUCTION IN SALMONELLA TYPHIMURIUM[1]

HARUO OZEKI[2]

Carnegie Institution of Washington, Department of Genetics
Cold Spring Harbor, New York

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IN the experimental transduction system involving *Salmonella typhimurium* and bacteriophage PLT-22 (ZINDER and LEDERBERG 1952), a number of instances of joint transduction of two (or more) markers have been observed (STOCKER *et al.* 1953; DEMEREC *et al.* 1954; DEMEREC *et al.* 1955). Thus it appears that the genetic material transferred by a phage particle can include more than one gene. Moreover, it has been found that only certain combinations of Salmonella markers can be transduced jointly: such markers are assumed to be linked on the chromosome. LENNOX (1955), working with a different system (*Escher*-ella markers can be transduced jointly: such markers are assumed to be linked genes in recombination experiments can be jointly transduced. It therefore seems reasonable to assume that the material transferred by a phage particle is itself a short fragment of the bacterial chromosome, as suggested originally by STOCKER and his co-workers.

The present study is concerned with the problem of whether or not the fragments carrying a given marker are uniform, that is, all of the same length and composition. The results reported here were obtained from observations of two different types of products appearing in transduction experiments made with auxotrophic markers, namely, (1) minute colonies, resulting from abortive transduction, which will be described in detail in a later section, and (2) large colonies, resulting from genetic recombination through transduction. The latter process will be referred to in this paper as "complete transduction," in contrast to "abortive transduction."

It has been thought that the genetic recombination involved in complete transduction results from crossing over between the chromosome of the recipient bacterium and a fragment of donor chromosome brought in by a phage particle (DEMEREC and DEMEREC 1956; LEDERBERG 1956; cf. MORSE *et al.* 1956); an even number of crossovers would be required to obtain a viable recombinant. The hypothesis that the material whose introduction results in abortive transduction is a fragment of chromosome has been discussed in detail by LEDERBERG (1956) and STOCKER (1956); their observations do not conflict with this hypothesis, and the additional information to be presented here supports the idea. A further assumption, based on the above hypothesis, will be made as a working premise,

[1] Aided by a grant from the American Cancer Society.

[2] Present address: The Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, S.W. 1, England.

viz., that the chromosome fragments whose introduction results in complete transduction (through crossing over) have the same size distribution as those concerned in abortive transduction.

MATERIALS AND METHODS

S. typhimurium LT-2 (ZINDER and LEDERBERG 1952) was the basic strain used in the experiments; it will be referred to as wild type. The auxotrophic strains were all derivatives of LT-2, and all were sensitive to the temperate phage PLT-22 (ZINDER and LEDERBERG 1952), which was the vector in the transduction experiments. The symbols (see DEMEREC 1956b) and growth requirements of the auxotrophic markers are listed below. Capital letters designate different loci within each category of requirement; numerals identify individual mutant strains, which are numbered in the order in which they were isolated.

(Since this paper was written, a uniform nomenclature for auxotrophic mutants has been proposed (Microbial Genetics Bulletin No. 16), and the symbols now in use for the mutants listed are as follows: adenine, *ade*; purine-plus-thiamine, *adth*; guanine, *gua*; histidine, *his*; methionine, *met*; and serine, *ser*.)

adC-2, *adC-7*, and *adE-11*: require adenine. (Guanine, hypoxanthine, and xanthine also satisfy this requirement.)

ad-3: requires adenine. (This mutant, although obtained in a single cycle of the penicillin screening procedure, behaves genetically as though it had undergone mutation at each of the loci *adA* and *adB*; see YURA 1956.)

athA-2, *athA-4*, *athB-6*, *athC-5*, *athC-14*, and *athD-12*: require one of the purines plus thiamine.

cysA-1, *cysB-12*, and *cys-5*: require cystine. (Mutant *cys-5*, although obtained in a single cycle, behaves genetically as a double mutant, *cysA-5 cysC-80*; see HOWARTH 1958).

guA-1: requires guanine.

hiA-38, *hiB-22*, *hiD-34*, and *hiD-39*: require histidine.

meA-22, *meB-45*, *meC-50*, and *meF-31*: require methionine.

proA-24 and *proA-46*: require proline.

se-1 and *se-5*: require serine.

tryA-8, *tryB-2*, and *tryD-10*: require tryptophan.

(For more details regarding these mutants, see DEMEREC *et al.* 1955, 1956.) The double-mutant strains were combinations of these markers, and their phenotypes were additive. They will be symbolized *tryA-8 cysB-12*, *athC-5 proA-46*, and so forth.

The minimal medium (M) contained K_2HPO_4, 10.5 gm; KH_2PO_4, 4.5 gm; $MgSO_4$, 0.05 gm; $(NH_4)_2SO_4$, 1.0 gm; sodium citrate, 0.47 gm; glucose, 2.0 gm; demineralized water, 1000 ml. For enriched minimal medium (EM), 0.01 percent dehydrated nutrient broth was added. Both media contained 1.5 percent agar for plate culture. The concentrations of various supplements added to the media were: adenine sulfate (A), 20 μg/ml; thiamine (B_1), 10 μg/ml; cysteine (C), 20 μg/ml; guanine (G), 20μg/ml; proline (P), 30 μg/ml; $Na_2S_2O_3.5H_2O$ (S), 1

mg/ml; tryptophan (T), 20 μg/ml. The supplemented media will be designated by combinations of the abbreviations shown above; for example, MT, minimal medium plus tryptophan; EMGP, enriched minimal medium plus guanine and proline.

In transduction experiments a suspension of the recipient bacteria was infected with phage grown on the donor bacteria in nutrient broth (for *cys* mutants, 20 μg/ml cysteine were added). After incubation at 37°C for ten minutes in broth or saline, to allow adsorption to take place, 0.1 ml of the suspension was plated on suitable medium (usually in triplicate). The number of infected cells plated was adjusted in each experiment to give a desirable number of colonies on the plates. Multiplicity of infection was always between one and ten (usually around five), to avoid lysis. Colonies were counted after one or two days' incubation at 37°C. The "minute colonies" were counted with a microscope at 10×–20× magnification (see DEMEREC and OZEKI 1958). Formulas for transduction experiments are written *strain* (recipient) × *strain* (donor).

When it was necessary to isolate phage-sensitive clones descended from transduced cells, a mutant strain (phage *H-4* of ZINDER) was substituted for the wild type PLT-22.

RESULTS

Abortive transduction among linked markers: It had been observed (OZEKI 1956) that, in certain transduction experiments with auxotrophic mutants of *S. typhimurium* LT-2, "minute colonies" appeared in addition to the large colonies expected as a result of complete transduction. Analysis of the mechanism of formation of such minute colonies showed that it is apparently similar to the mechanism responsible for the formation of "trails" in the transduction of motility in Salmonella—namely, abortive transduction (STOCKER *et al.* 1953). Evidence indicates that in abortive transduction the fragment introduced into a recipient cell fails to be incorporated into its genome, and does not duplicate, but persists in a functional state in one of the descendants through a number of generations (see also STOCKER 1956; LEDERBERG 1956). The formation of minute colonies when auxotrophic recipient cells are plated on minimal medium requires the presence in some of these cells of genetic fragments containing the wild type allele (functional unit) corresponding to the mutated locus in the recipient strain. Consequently, the appearance of minute colonies on a plate that does not contain the growth-factor requirement corresponding to the marker being studied is a sign of the transfer of the wild type allele of that locus by the transducing phage particle (OZEKI 1956; DEMEREC and OZEKI 1958).

To extend the above described observations, the formation of minute colonies was examined in experiments with double-mutant recipients whose two markers are linked in transduction. The double mutants *tryD-10 cysB-12* and *tryA-8 cysB-12* were used, since linkage among these three markers has been clearly demonstrated (DEMEREC and Z. HARTMAN 1956). MS served as "minimal" medium for these two-marker mutants because *cysB* mutants accumulate the

growth factor for any *try* mutant on M or EM medium, and the addition of thiosulfate suppresses this accumulation without supporting the growth of *cysB-12* cells and without inhibiting the growth of wild type cells. One set of results obtained in the experiments with *tryD-10 cysB-12* is shown in Table 1. Similar results were obtained with *tryA-8 cysB-12* as recipient.

TABLE 1

Abortive transduction with tryD-10 cysB-12 *recipient bacteria. Figures represent the numbers of minute colonies per plate (average of three plates) after 18 hours of incubation at 37°C. Figures in parentheses are the numbers of large colonies (results of complete transductions) on the same plates*

Medium	Donor			
	tryD+ *cysB*+	*tryD-10 cysB*+	*tryD*+ *cysB-12*	*tryD-10 cysB-12*
MS*	4700 (122)	0 (0)	0 (0)	0 (0)
MT	4300 (601)	4100 (399)	0 (0)	0 (0)
MC	4000 (355)	0 (0)	2350 (135)	0 (0)

* MS served as "minimal" medium in this experiment (see text).

The formation of minute colonies on MS plates required the presence in recipient cells of fragments containing both wild type alleles, *tryD*+ and *cysB*+, whereas the formation of minute colonies on MT and MC plates required the presence of only one of these two wild type alleles. It appears that most, if not all, of the fragments carrying the *tryD* locus also carried the *cysB* locus, and vice versa; for the numbers of minute colonies observed on MS, MT, and MC were not significantly different within any given set of experiments with *tryD*+ *cysB*+ (wild type) as donor. This conclusion is supported by results of experiments (see Table 5) in which *tryD-10*, *cysB-12*, and *tryD-10 cysB-12* bacteria were all infected with the same phage preparation from a wild type donor; the numbers of minute colonies were similar.

These results suggest two possibilities concerning the nature of the chromosome fragments participating in abortive transduction: (1) the fragments are long in relation to the distance between the *tryD* and *cysB* loci, and the chromosome of donor cells is disrupted at random; or (2) the fragments are short, but chromosome fragmentation does not occur in a random manner and the fragments concerned always or almost always include both the *tryD* locus and the *cysB* locus.

Since the *try-cysB* mutants are the only linked double mutants showing abortive transductions that we have isolated so far, the same technique could not be applied to other regions of the chromosome to extend the observations described above. However, similar results have been obtained in experiments involving *athD-12* and *athB-6*. These two genes are rather closely linked, about 85 percent donor type and 15 percent wild type being recovered in *athD-12* (recip.) × *athB-6* (donor) tests. In abortive-transduction experiments with *athD-12* as recipient, smaller minute colonies (less than half the diameter) were observed on M (or

EM) plates when *athB-6* was donor than when wild type was donor. The numbers of minute colonies appearing in the experiments were approximately the same with the two donors, and the size distribution curves were in both cases unimodal (the modes of these two curves differed by a factor of two). This result suggests that all or almost all fragments carrying *athD*$^+$ also carry *athB-6* or *athB*$^+$. The reduction in size of minute colonies when the *athB-6* marker is present has not been explained.

The formation of minute colonies has also been tested with unlinked double mutants as recipients, that is, double mutants from which stable prototrophs had not been obtained in experiments with wild type donor bacteria. Ten different unlinked double mutants were tested with phage grown on wild type bacteria. They were *cysA-5 cysC-80*, *ad-3* (*adA adB*), *athA-2 meF-31*, *adE-11 meB-45*, *hiB-22 tryA-8*, *tryB-2 meA-22*, *adE-11 hiA-38*, *athC-5 proA-46*, *athA-2 hiD-34*, and *cysB-12 proA-24*. With most of the strains no minute colonies were observed on minimal plates. With some, minute colonies did appear; but in every case they were shown to be the result of abortive transduction at one locus, the other locus being "leaky."

These results show that the relationship (linkage or its absence) among auxotrophic markers inferred from observations of complete transduction is maintained in abortive transduction. It furthermore appears that, in the only two systems testable, pairs of genes which are inferred to be linked are generally transferred together in abortive transduction. These observations, together with the fact that in complete transduction linkage between markers whose phenotypes are not closely related is very rare, suggest that the fragments participating in transduction are generally short, and support possibility (2) above, rather than possibility (1). In this they also agree with the results of an earlier analysis by DEMEREC and Z. HARTMAN (1956) of complete transduction of *try cysB* double mutants by wild type (Table 2), which showed that the number of two-marker

TABLE 2

Average numbers of colonies per experiment of the different transduction classes recovered in experiments with try cysB × try$^+$ cysB$^+$. *Media used: M=minimal; MT=minimal with tryptophan added; MC=minimal with cysteine added (From DEMEREC and Z. HARTMAN 1956)*

Recipient	M	MT		MC	
	try$^+$ *cys*$^+$	*try*$^+$ *cys*$^+$	*try cys*$^+$	*try*$^+$ *cys*$^+$	*try*$^+$ *cys*
tryA-8 cysB-12	150	123	224	261	312
tryB-2 cysB-45	253	234	935	213	272
tryD-10 cysB-12	336	337	1,512	351	877
tryD-11 cysB-18	143	141	523	193	391

transductions was always smaller than either of the single-marker classes, and thus indicated that the genetic lengths of the regions beyond these two genes on a fragment are not greater than the distance between them.

Reciprocal transduction: Only a few combinations of recipient and donor strains are known in which donor-type recombinants can be recovered in both directions of reciprocal experiments. One such combination, *athC-5* and *guA-1* has been studied in detail.

Linkage of the three loci *adC*, *athC*, and *guA* has been reported previously (DEMEREC *et al.* 1956). Results of reciprocal transductions among *adC*, *athC*, and *guA* mutants, presented in Table 3, suggest that the order of these loci is *adC–athC–guA*. Usually, numbers of colonies obtained in different transduction experiments cannot reliably be compared, since frequency of transduction is affected by many factors that are difficult to control. However, the ratios of wild type (one-marker) to donor type (two-marker) transductions in different experiments can probably be compared. It was originally thought that, the chromosomes of donor bacteria being disrupted into small fragments in a random way, these ratios simply reflected the distance between the two markers involved: the closer the markers the higher the probability that both donor markers would be incorporated. On this hypothesis, the ratio of wild type to donor-type transduction would always be the same for a particular pair of markers, in whichever direction the transduction was carried out, because the distance between the two markers and the mean length of the transducing fragments would be the constant factors. But in the present case the ratios varied according to the direction of transduction. For instance, with *guA-1* as recipient and *athC-5* as donor, the ratio of wild type to donor-type was 2.9 to 1, but with *athC-5* as recipient and *guA-1* as donor the ratio was 0.9 to 1, as shown in Table 3. To find out whether the difference in the ratios was due to the difference in the wild type class or to the difference in the donor-type class, the following experiments were done.

A spontaneous proline-requiring mutant of *athC-5* (designated *athC-5 proA-*

TABLE 3

Results of reciprocal transduction experiments with adC, athC, *and* guA *mutants. Figures represent average numbers of colonies per plate (based on three plates), expressed as wild type/donor type. Figures in parentheses show the ratios of wild type to donor type. Number of infected bacteria per plate, about* 2×10^8*; multiplicity of infection, about five. Medium used was enriched minimal supplemented where possible with a growth factor supporting the multiplication of donor-type bacteria without allowing growth of the recipient cells*

	Donor				
Recipient	*adC-2*	*adC-7*	*athC-5*	*athC-14*	*guA-1*
adC-2	0/–	3/–	181/–	163/–	156/–
adC-7	4/–	0/–	196/–	130/–	189/–
athC-5	322/38 (8.5)	311/49 (6.3)	0/–	10/–	155/170 (0.9)
athC-14	267/42 (6.4)	251/46 (5.5)	11/–	0/–	144/129 (1.1)
guA-1	426/12 (35.5)	490/13 (37.7)	508/174 (2.9)	540/154 (3.5)	0/–

46) was first isolated. Then the *proA-46* marker was combined with *guA-1* by infecting *athC-5 proA-46* with phage from *guA-1* and isolating the *athC*$^+$ *guA-1 proA-46* recombinant (nonlysogenic). To analyze the relation between frequencies of complete transductions at the *athC-guA* region and at the *proA* region, both two-marker strains, *athC-5 proA-46* and *guA-1 proA-46*, were infected with phage grown on wild type bacteria. As shown in Figure 1, a linear relation was observed between frequencies of transduction in the two regions within a certain range of multiplicity of infection. Similar results were obtained with *adC-7 proA-46* (nonlysogenic), isolated in the transduction *athC-5 proA-46* (recip.) × *adC-7* (donor).

Thus corrections can be made for numbers of transductions at the *athC-guA* region in different experiments on the basis of the numbers of colonies resulting from complete transductions from *proA-46* to *proA*$^+$ in the same experiments. This method was applied to the results of reciprocal experiments between *athC-5* and *guA-1* (Table 4). As the table shows, the frequencies of transductions to the donor type (two-marker transductions) were similar in the two experiments, whereas the frequencies of one-marker transductions were significantly different. As a result, the ratios of one-marker to two-marker transductions are different for the two directions of the reciprocal experiments. These experiments were repeated several times, using different phage preparations, with similar results; two sets of data are given in Table 4. The difference in ratios may be explained on the assumption that all transducing fragments carrying the *athC-guA* region are approximately identical, and that the position of a marker on the fragment has an important effect on the probability of its incorporation into the recipient chromosome. Figure 2 is a schematic representation of the positions of the *athC-5* and *guA-1* sites on the transducing fragments, based on the values presented in Table 4. In class c of the table, the similar values obtained for the reciprocal experiments 1 and 2 are explained on the basis that in both cases crossing over in the X and in the Z portion of the fragment was required for incorporation of the *athC* and *guA* loci; and the difference in values in class b is explained on the basis

TABLE 4

Reciprocal transduction experiments with linked markers. Each figure represents the actual number of colonies on a total of three plates. Figures in parentheses are corrected values (per 1000 colonies in column a). (a). Transduction from proA-46 *to* proA$^+$. *(b). One-marker transduction: expt. 1, from* athC-5 guA$^+$ *to* athC$^+$ guA$^+$; *expt. 2, from* athC$^+$ guA-1 *to* athC$^+$ guA$^+$. *(c). Two-marker transduction: expt. 1, from* athC-5 guA$^+$ *to* athC$^+$ guA-1; *expt. 2, from* athC$^+$ guA-1 *to* athC-5 guA$^+$

Expt.	Recipient	Donor	a	b	c	b/c
1	*athC-5 guA*$^+$ *proA-46*	*athC*$^+$ *guA-1 proA*$^+$	1072 (1000)	167 (156)	182 (169)	0.93
			684 (1000)	134 (196)	138 (202)	0.97
2	*athC*$^+$ *guA-1 proA-46*	*athC-5 guA*$^+$ *proA*$^+$	2,149 (1000)	1,315 (612)	399 (186)	3.29
			724 (1000)	454 (628)	144 (198)	3.16

that experiment 1 involved the X and Y portions whereas experiment 2 involved Y and Z. Thus the difference in ratios shown in Table 4 (see also Table 3) can be explained on the assumption that the Z portion is longer than the X portion in

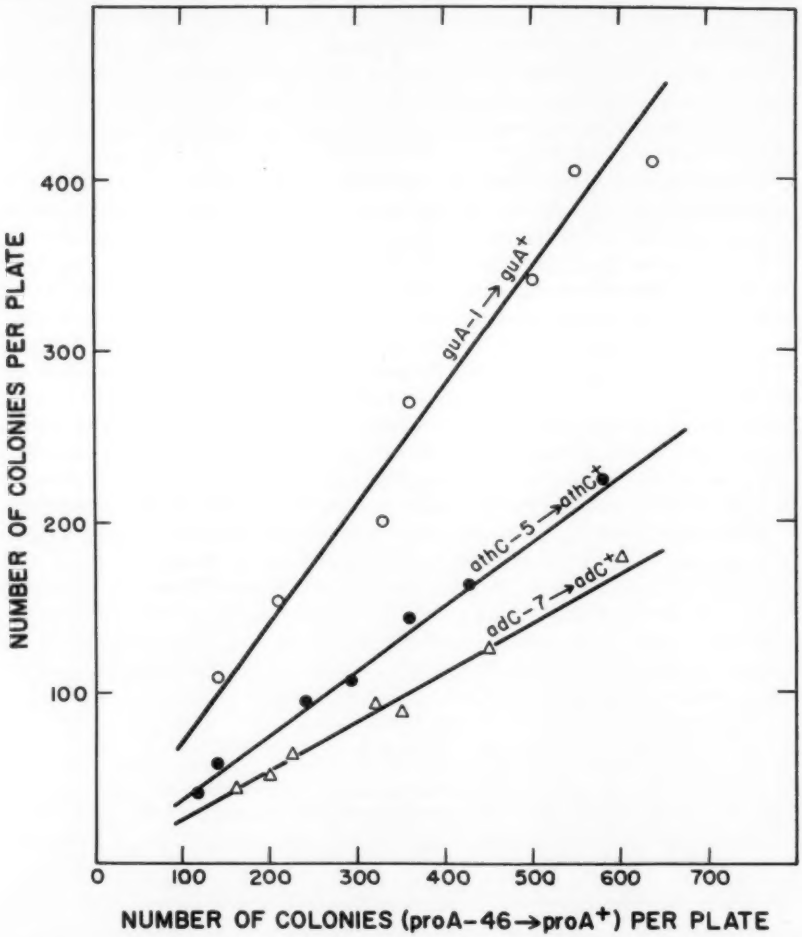

FIGURE 1.—Relationships between frequencies of complete transduction occurring at the *adC–athC–guA* region and the *proA* region. Data were obtained in the following transduction experiments: *guA–1 proA–46* (recip.) × wild type (donor), platings on EMG and EMP media; *athC–5 proA–46* (recip.) × wild type (donor), platings on $EMAB_1$, and EMP; *adC–7 proA–46* (recip.) × wild type (donor), platings on EMA and EMP. To obtain a range of numbers of colonies per plate, the number of bacteria plated was varied from 10^8 to 2×10^8, and the multiplicity of infection from one to ten.

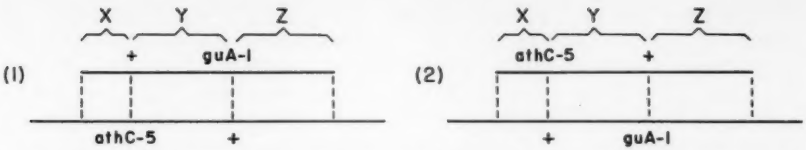

FIGURE 2.—Schematic representation of locations of the genetic markers in the *athC-5–guA-1* reciprocal transductions. (1) and (2) correspond to experiments 1 and 2 in Table 4. Lower line represents the bacterial chromosome, upper line the transducing fragment. X, Y, and Z: relative distances between genetic markers and terminal points on the transducing fragment.

Figure 2; that is, the fragmentation of the donor chromosome does not occur in a random way.

When *athC-5* is infected with phage grown on wild type bacteria, the incorporation of the *athC-5*$^+$ marker into a viable recombinant requires one crossover in the X portion and one in the Y or Z portion, so that the probability of incorporation, in cells which have received the chromosome fragment carrying *athC-5*$^+$, is $X(Y + Z)$. Consequently, the ratio of frequencies(f) of transduction to prototrophy in *athC-5* and in *guA-1* recipients with wild type donor is expressed as f *athC*/f *guA* $= X(Y + Z)/Z(X + Y)$. The relative values of X, Y, and Z may be estimated from one set of the b/c ratios in Table 4, as follows: $Y/Z = 0.93$, and $Y/X = 3.29$; accordingly $X/Y/Z = 0.93/3.05/3.29$; whence f *athC*/f *guA* $= 1/2.23$. Similar calculations, based on the data from further tests of the same kind as those recorded in Table 4, gave ratios of f *athC* to f *guA* in the range of 1/1.6 to 1/2.5. The ratios observed in the experiments described in the next section fell within the range that agreed well with these calculated values. The agreement between predicted and experimental values lends further support to the hypothesis that the ends of transducing fragments are predetermined.

As mentioned above, the order of the loci *adC*, *athC*, and *guA* is *adC–athC–guA*; that is, *adC* lies in the X portion of Figure 2. In experiments with *adC-7* and *athC-5*, donor-type recombinants could be scored only when *adC-7* was the donor. In this experiment the ratio of wild type to donor-type transduction was 6.3 to 1 (Table 3); by an argument similar to that above it is inferred that *adC-7* lies at a point such that its distance from the left end of the transducing fragment (Figure 2) is 1/6.3 of its distance from *athC-5*. The predicted value of f *adC*/f *athC* is 0.15/1, and the experimental one is 0.77/1 (see next section). In this case the experimental value for *adC* is about five times higher than the expected one, although there is still a tendency for the frequency of complete transduction to decrease towards the end of the fragment. This disagreement might be explained by assuming the existence of some mechanism that would increase the probability of incorporation of the marker *adC-7*$^+$. It is possible, however, that the method used for calculating the distances between markers and the ends of fragments was inaccurate.

The results presented in Table 2 for the one-marker (*try*$^+$ or *cysB*$^+$) and two-marker(*try*$^+$ and *cysB*$^+$) classes, in transduction experiments involving various

combinations of *try* and *cysB* as recipients and the wild type as donors, show the same type of differences between the frequencies of the two single-marker classes as has already been described for transduction at the *athC-guA* region. In the case of *tryA-8 cysB-12*, the difference between the one-marker classes may not be significant, since the variation in numbers of prototrophs (two-marker class) on the different types of plates indicates that some complicating factor may have been present.

Relative frequencies of transduction among linked markers: Relative frequencies of complete transductions of *adC-7*, *athC-5*, and *guA-1* markers by phage grown on wild type bacteria were measured in two different ways. (1) The marker *proA-46* was used as a standard, as described above; the results are shown in Figure 1. (2) Strains carrying a single marker—*adC-7*, *athC-5*, or *guA-1*—were infected with equal amounts of phage from the same wild type donor preparation (Table 5). The two sets of data are in agreement, and indicate the following relative frequencies of complete transduction:

$$f\ adC\text{-}7\ :\ f\ athC\text{-}5\ :\ f\ guA\text{-}1 \approx 1\ :\ 1.3\ :\ 2.5$$

This agreement between results obtained by different methods seems to indicate that the difference in frequencies of complete transduction among these three markers is not due to physiological differences among the recipient cells with respect to adsorption rate, residual growth rate, or the like.

On the other hand, the frequencies of abortive transduction for these markers were similar. Accordingly, the ratios of abortive to complete transduction varied inversely with the frequency of complete transduction (Table 5).

Relative frequencies of transduction among unlinked markers: Since frequencies of transduction of different markers have been known to vary over a wide range (order of 10^{-5} to 10^{-7} per phage), it may be of some interest to compare frequencies of complete transduction and ratios of abortive to complete transduction for several unlinked markers (Table 6). The results are different from those obtained with linked markers; there is no inverse relation between the frequency of complete transduction and the ratio of abortive to complete

TABLE 5

Relative frequencies of abortive and complete transduction of linked markers in three different chromosome regions. Recipient strains whose markers are linked are grouped together. Donor, wild type. Platings on minimal medium. AT/P=number of minute colonies per 4×10^8 phage particles; CT/P=number of large colonies per 4×10^8 phage particles; AT/CT=ratio of abortive to complete transductions

	Recipient							
	adC-7	*athC-5*	*guA-1*	*tryD-10*	*cysB-12*	*tryD10 cysB-12*	*se-1*	*se-5*
AT/P	1,120	1,240	1,030	3,810	3,430	3,070	1,440	1,260
CT/P	81	105	190	346	503	136	160	152
AT/CT	13.7	11.8	5.4	11.0	6.8	22.5	9.0	8.3

TABLE 6

Relative frequencies of transduction among unlinked markers. Donor, wild type; platings on minimal medium. AT/P=numbers of minute colonies per 4×10^8 phage particles; CT/P=numbers of large colonies per 4×10^8 phage particles; AT/CT= ratio of abortive to complete transductions

	Recipient					
	hiD-39	*athA-4*	*proA-46*	*cysA-1*	*meC-50*	*athD-12*
AT/P	18,880	7,160	1,760	1,700	900	480
CT/P	1,988	664	376	188	64	25
AT/CT	9.5	10.8	4.7	9.0	14.0	19.2

transduction among unlinked markers. This will be discussed in the following section.

DISCUSSION

The results presented indicate that the fragments participating in abortive transduction at one region of Salmonella chromosome, which has been closely examined, always carry a restricted but constant group of markers within which simultaneous complete transduction of markers can be demonstrated. Moreover, in any tests made so far, unlinked markers (so inferred from observations of complete transduction) have never been carried jointly by a fragment participating in abortive transduction. These observations suggest that in abortive transduction, as well as in complete transduction, the transfer of genes and not the products of genes is involved. A similar case has been reported by LEDERBERG (1956), who observed the uniform coupling of a locus (for formation of flagella) with another linked locus (for flagellar antigen type) in fragments participating in abortive transduction.

Fragments concerned in complete transduction, in two different regions tested, have been shown to have a nonrandom distribution of markers with relation to the ends of the fragment. Since all, or almost all, markers appear to be readily transducible, it seems probable that the chromosomes of donor bacteria are disrupted into small fragments having a more or less definite size and distribution of markers. Once the chromosome is broken down in this regular way, the fragments produced can be picked up by phage particles and transferred to recipient cells.

Other explanations of the uniformity of the fragments are also possible, however, and cannot be excluded. It is known that in *Escherichia coli* all those particles of lambda phage which are capable of bringing about transduction of the *Gal* loci are defective, lacking a particular region of the phage chromosome (ARBER *et al.* 1957). This suggests the possibility that, in Salmonella transduction also, phage particles can carry only particular regions of the bacterial chromosome, which have an affinity for or genetic homology with particular parts of the phage genome. Some evidence of partial genetic homology between phage PLT-22 and *S. typhimurium* LT-2 has been reported by GAREN and ZINDER (1955).

The hypothesis introduced here, that the ends of transducing fragments are

predeterminate, may explain the following cases. As was mentioned earlier, the *ad-3* mutant was obtained in a single cycle of the penicillin screening procedure (LEDERBERG 1950) but behaves genetically as though it had undergone mutation at each of the loci *adA* and *adB*, although these loci are unlinked as far as can be judged from (complete) transduction experiments (YURA 1956). Similarly, *cys-5*, although obtained in a single cycle of isolation, behaves as a double mutant, *cysA cysC*; and no linkage between the *cysA* and *cysC* loci has been demonstrated by transduction (HOWARTH 1958). Since there is no double mutant, isolated in a single cycle of isolation and requiring two different amino acids, in DR. DEMEREC's collection of bacterial mutants including more than five hundred auxotrophs of *Salmonella typhimurium*, there is almost no chance of obtaining a mutant having two independent mutations at two adenine (or two cystine) loci during a single cycle of penicillin screening. It may, therefore, be suggested that in these mutants a mutation extends over a certain length of chromosome, covering at least a part of each of the loci involved, as has been reported in several other instances in this organism (DEMEREC 1956a; CLOWES 1958). This implies that two functionally related loci *adA* and *adB* (or *cysA* and *cysC*) are closely linked on the chromosome, as has been reported in many other instances by DEMEREC (1956a). The discrepancy between this consideration and the conclusion arrived at by the transduction experiments (absence of linkage) suggests that there is a point, in a region involving *adA* and *adB* (or *cysA* and *cysC*), where breakage of the chromosome prior to transduction usually occurs. In this relation a case reported by CLOWES (1958) may be considered. Two cystine-requiring mutants of *S. typhimurium*, *cys-48* and *cys-50*, classified as *cysD* mutants by phenotype, behaved in transduction tests as though not linked to any other markers of the *cysD* group. It is, of course, difficult to decide whether or not *cys-48* or *-50* belongs to the *cysD* locus on these grounds alone. However, since in Salmonella there is a strong tendency for genes controlling related reactions to be grouped close together on the chromosome (DEMEREC 1956a), this case may be thought of as another example suggesting predeterminate fragmentation of chromosomes.

The differences in frequencies of complete transduction observed among linked markers seem to reflect differences in the distances between the markers and the ends of the fragment. The frequencies of abortive transduction are roughly the same among these linked markers. Unlinked markers show more striking differences in complete-transduction frequencies, which are paralleled, although very roughly, by their frequencies of abortive transduction (see Tables 5 and 6). Thus it appears that the frequency of complete transduction of a given marker is affected not only by the probability of its incorporation but also by the availability of the region containing it. ZINDER (1955) has shown that phage populations obtained at various times after infection of donor cells have different capacities to transduce given markers. This variability presumably reflects differences in the availability of fragments carrying these markers at different times during the course of phage growth, and may indicate that disruption of the donor chromosome occurs in some sequential manner.

SUMMARY

Pairs of markers which can be simultaneously transduced (complete transduction), and which are inferred to be linked, are always (or almost always) present together in the fragments participating in abortive transduction of one or the other marker. Other, unlinked, markers, according to tests made so far, are never carried by a fragment participating in abortive transduction of one marker.

In transduction experiments $A^-\ B^+$ (recip.) $\times$ $A^+\ B^-$ (donor) and the reciprocal $A^+\ B^-$ (recip.) $\times$ $A^-\ B^+$ (donor), the frequencies of complete transduction of the two single markers, A^+ and B^+, are not the same whereas the frequencies of two-marker transduction (A^+B^- or A^-B^+) are about equal in the two reciprocal combinations. These results may be explained by the hypothesis that complete transduction is the consequence of crossing over between the chromosome of the recipient and a transduced fragment of donor chromosome of uniform composition, the probability of incorporation (complete transduction) for any particular marker varying according to its position in relation to the ends of the fragment.

Among linked markers, the frequency of occurrence of complete transduction varies, whereas frequencies of abortive transduction for these markers are similar. Among unlinked markers, the differences in frequency of complete transduction are much greater, and the frequencies of abortive transduction roughly parallel those of complete transduction.

The hypothesis that the chromosomes of donor bacteria are broken up into fragments of predeterminate composition prior to transduction is considered.

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GENETIC MECHANISM OF MATERNAL EFFECT FOR TUMOROUS HEAD IN DROSOPHILA MELANOGASTER[1]

ELDON J. GARDNER

Utah State University, Logan, Utah

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A MATERNAL effect hypothesis was established to explain a difference in the results of reciprocal crosses between tumorous head (*tu-h*) flies and those from "wild" and mutant laboratory stocks (GARDNER and WOOLF 1949). Two major genes were associated with the phenotype, a sex-linked recessive *tu-1* postulated to control the maternal effect and a third chromosome semidominant *tu-3* which is basic and necessary for any expression of the trait. Modifiers and alleles of *tu-1* and modifiers of *tu-3* were found in laboratory stocks (GARDNER and STOTT 1951) and in natural populations (GARDNER, STOTT and DEARDEN 1952). Samples from 28 natural populations from widely separated geographical locations were collected and cultured in the laboratory (GARDNER and GARDNER 1953). Individual flies from the first generation of these samples were crossed reciprocally with *tu-h*. The F_1 results from the reciprocal crosses were different, and the data were interpreted as further evidence for a maternal effect.

From linkage data (STOTT and GARDNER 1952) *tu-1* was located near forked (*f* 56.7) in the first chromosome and *tu-3* was located at 58 in the third chromosome. The position of *tu-1* was later revised on the basis of more extensive linkage data and placed at 64.5, near the heterochromatin region of the first chromosome. Several questions have arisen concerning the action of the two major genes and the nature and action of their modifiers. The Y chromosome is known to carry or act as a major modifier of the maternal effect. MAYEDA (in press) has shown that the Y chromosome, carried by attached-X *tu-h* females which are mated to wild males, increases the expression of the trait from about 35 percent to more than 60 percent. Chromosome inversions have also been found to increase the expression of the trait. The influence of these and other chromosome aberrations is being investigated.

In the summer of 1957 PROFESSOR RICHARD GOLDSCHMIDT kindly reviewed all of the data available and suggested that the maternal effect hypothesis should be tested more critically with special reference to the genetic mechanism and a possible cytoplasmic factor. Such a test is especially appropriate because the *tu-h* stock is now being used in biochemical and developmental studies in which the maternal effect hypothesis has particular significance.

The following experiments were designed to determine whether the difference

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in the results of reciprocal crosses is due to a maternal effect or a cytoplasmic factor and whether the genetic mechanism can be explained by the segregation of a single recessive gene. Maternal effect is defined here as the direct influence of the mother's genes in predetermining the expression of the trait in the progeny. A cytoplasmic factor would be expected to persist in the maternal line through cytoplasmic transmission from affected mothers to their progeny.

METHODS AND MATERIALS

The first experiment was designed as a series of backcrosses from which the predicted results based on the hypothesis could be tested. Reciprocal crosses were made between *tu-h* flies and those from a highly inbred Oregon-R stock. F_1 females, not showing the tumorous head expression, were taken from each of the reciprocal crosses and backcrossed to *tu-h* males. Successive backcrosses to *tu-h* males were continued from the progeny of the reciprocal crosses for seven generations. Females not showing the trait were taken from the progeny of the seventh backcross and crossed with Oregon males. A total of eight backcrosses involving females not showing the trait was thus included. In the first seven *tu-h* was used as the male recurrent parent and in the eighth Oregon was used.

At the conclusion of these eight backcrosses involving normal appearing females, abnormal females were selected from the backcross cultures and carried through a series of two successive backcrosses to Oregon males. As a final check, Oregon females were crossed with abnormal males from the seventh backcross progeny.

The *tu-h* stock used in the first experiment was the standard laboratory stock which had been maintained for about ten years in mass matings by selecting for each generation several virgin females and young males showing the trait. The average penetrance at room temperature during the period of the experiment was 79 percent. The Oregon-R stock had been maintained by pair matings for 225 generations except for three mass matings which were made in generations 116, 151, and 165.

The second experiment was similar to the first except that a different *tu-h* line was used and an inbred Canton-S stock was used instead of Oregon-R for the outcrosses. In this experiment only two successive backcrosses were made to *tu-h* and two to Canton as the recurrent parent. The *tu-h* line was similar to that described by SIMMONS and GARDNER (1958). It was highly selected and had been maintained from pair matings rather than mass cultures. Average penetrance was 93 percent with a range from 83 to 96 percent. Presumably modifiers were accumulated by rigorous selection and made homozygous through intense inbreeding. The Canton-S stock was made isoallelic by use of markers in 1939 and had been maintained in mass cultures since that time.

Earlier experiments (GARDNER and WOOLF 1950; GARDNER and RATTY 1951) had shown that the tumorous head phenotype is very sensitive to minor environmental influences and genetic modifiers. When the flies from the standard inbred *tu-h* stock were outcrossed individually with those from laboratory stocks and

with samples from natural populations a good deal of individual variation was observed among cultures which were presumed to be similar. This was attributed to differences in the interbottle environment and genetic modifiers carried by some flies. In the present experiment only inbred lines were used and the external environment was controlled as well as possible. Considerable variation was observed from culture to culture in spite of all the care which was taken in conducting the experiments.

The experimental cultures were maintained at 25±1°C in half-pint milk bottles on the standard cornmeal, molasses, agar medium to which live yeast was added. Only pair matings were used for the experiments. Parents were removed eight days after the mating, and the progeny were classified each day or two until the 18th day after the mating. The classification was made by separating the males and females showing the trait from those not showing the trait. To save printing space only total percentages of abnormal flies are included in the tables with accuracy to the nearest whole number. Where crosses are symbolized, the female parent is always written first.

EXPERIMENTAL RESULTS

The results from the first experiment are summarized in Tables 1 and 2. Percentages of abnormal flies in the *tu-h* stock cultures from which the reciprocal crosses were made are given in the first column. The average of ten cultures was 79 percent with a range from 66 to 91 percent. Next, the F_1 results are summarized

TABLE 1

Crosses between tu-h *females and Oregon males, seven successive backcrosses to* tu-h *males (a to g) and one to Oregon males (h) followed by two successive backcrosses to Oregon males (j, k)**

			Percent abnormal progeny from pair matings										
			Normal appearing female from culture with three percent or less							Abn from efg × *tu-h*	Abn from g × Oregon	Abn from h, j × Oregon	Oregon × *tu-h* from g
			× *tu-h*					× Oregon					
Inbred *tu-h*	F_1	+F_1 × *tu-h* a	b	c	d	e	f	g	h	i	j	k	l
81	26	1	0	2	1	1	0	62	0	70	9	0	0
85	47	0	1	2	0	0	40	0	0	93	39	0	0
70	53	0	22	3	78	79	19	40	0	81	35	0	0
79	46	0	0	1	..	2	88	0	0	78	19	0	0
66	38	1	0	3	..	55	26	1	24	66	38	0	0
82	20	0	0	74	..	30	0	0	18	76	40	0	0
88	26	0	12	..	..	45	68	33	23	59	24	1	0
79	23	0	78	..	..	2	0	2	24	6	38	0	0
91	33	0	56	..	..	77	16	50	36	64	..	0	0
74	16	0	63	..	..	49	..	100	0	52	..	0	0
Ratio (from entire experiment): Heterozygous to homozygous mothers from cultures with heterozygous mothers			9:9	5:1	2:1	4:7	3:6	8:11	11:13				

* To save space only the first ten percentages are given in each column.

TABLE 2

Crosses between Oregon females and tu-h *males, seven successive backcrosses to* tu-h *males (a to g) and one to Oregon males (h) followed by two successive backcrosses to Oregon males (j, k)**

				Percent abnormal progeny from pair matings									
				Normal appearing female from culture with more than 30 percent						Abn from efg × *tu-h*	Abn from g × Oregon	Abn from h, j × Oregon	Oregon × *tu-h* from g
				× *tu-h*				× Oregon					
Inbred *tu-h*	F_1	+F_1 × *tu-h* a	a × *tu-h* b	c	d	e	f	g	h	i	j	k	l
81	0	0	50	47	10	27	29	50	18	44	30	0	0
85	0	0	34	33	23	20	84	54	12	74	51	0	0
70	0	0	49	50	43	34	87	81	11	85	54	0	0
79	0	0	1	44	62	79	53	60	32	50	43	0	0
66	0	0	0	..	51	70	84	69	51	75	58	0	0
82	0	0	82	..	27	63	77	83	9	76	41	0	0
88	0	0	43	..	..	74	58	85	39	87	80	0	0
79	0	0	42	..	..	76	91	76	24	78	41	0	0
91	0	0	0	..	..	71	72	..	16	..	40	0	0
74	0	0	52	..	..	..	57	..	20	..	61	0	0

Ratio (from entire experiment): Heterozygous to homozygous mothers from cultures with heterozygous mothers

9:21

* To save space only the first ten percentages are given in each column.

showing the characteristic difference from reciprocal crosses. From the 13 pair matings between *tu-h* females and Oregon males (Table 1) the average percentage of abnormal flies was 32 with a range from 16 to 53 percent. No abnormal flies were observed in the 20 cultures from pair matings between Oregon females and *tu-h* males (Table 2).

From the first backcross (column a, Tables 1 and 2) between normal appearing females and *tu-h* males from the inbred stock the results from the two original reciprocal crosses were similar. None of the cultures produced more than one percent of abnormal flies. All mothers were heterozygous for *tu-1* and the small percentage was attributed to the independent action of *tu-3*.

In the next six backcrosses (columns b-g) between normal appearing females from each preceding backcross culture and *tu-h* males from the inbred stock there is evidence for segregation of *tu-1*. This is also true of the next backcross to Oregon males (column h). When females were taken from cultures with only small percentages of abnormal flies (0–3 percent), presumed to be heterozygous for *tu-1*, about half of the cultures produced small percentages and half produced about 30 percent or more. Those with about 30 percent or more were presumed to have come from mothers homozygous for *tu-1* and those with small percentages were presumed to have arisen from heterozygous mothers. All backcross progeny had at least one *tu-3* which came from the *tu-h* male. Some, presumably those in cultures with higher percentages of abnormal flies, also received a *tu-3* from the

mother. Expressions higher than about 30 percent in individual cultures were attributed to an additional *tu-3* gene from the mother or modifiers.

At the bottom of Tables 1 and 2 the ratios are given for the numbers of cultures with low percentages of abnormal flies and those with about 30 percent or more. Those included are only from parent cultures with low percentages. The mothers were presumably heterozygous for *tu-1*. On the basis of the hypothesis, half of the cultures from normal appearing females taken from backcrosses between heterozygous females and *tu-h* males should have about 30 percent or more of abnormal flies and half should have three percent or less. From the total of 120 such cultures 69 had about 30 percent or more and 51 had three percent or less. The chi-square test for goodness of fit based on the 1:1 expectation showed: $\chi^2 = 2.70$. $P = .10$ to .20. The difference between the expectation and the experimental result can be explained by chance.

When females were taken from cultures having about 30 percent or more of abnormal flies, i.e., homozygous for *tu-1*, all of the cultures produced about 30 percent or more of abnormal flies. The increase above 30 percent was attributed to the number of *tu-3* genes and modifiers present in the progeny. The proportion of abnormal flies increased in the successive backcrosses presumably because some of the *tu-3* genes became homozygous in the females from inbreeding and modifiers were accumulated. At the end of the seventh backcross to *tu-h* males, in the series originating from the cross between *tu-h* females and Oregon males (Table 1), there were some females which were not yet homozygous for *tu-3*. This was expected because selection of females was continuously against the abnormality. In the series originating from the cross between *tu-h* females and Oregon males, heterozygous females were mated in each generation and segregation of *tu-1* was continued through the seven backcrosses (columns b-h). From a total of 103 matings 90 were made from cultures with low percentages and gave results expected from heterozygous mothers. Thirteen were made from cultures with about 30 percent or more and behaved as expected for homozygous mothers.

In the series from the reciprocal cross between Oregon females and *tu-h* males evidence of segregation of *tu-1* was observed in the second backcross (column b). The four females for the third backcross were taken from a single culture in which *tu-1* was presumably homozygous. All of the 50 succeeding cultures in the series (columns c to h) behaved as expected for mothers homozygous for *tu-1*. The 25 cultures (column h) from presumably homozygous mothers were similar to the F_1 from the original cross between *tu-h* females and wild males. The average percent of abnormal flies was 25 with a range from nine to 51 percent. In the 8 cultures (column g) with similar mothers but with *tu-h* for the recurrent parent an average of 70 percent of abnormal flies was produced with a range from 50 to 85 percent. This difference was attributed to the *tu-3* genes introduced by the males, modifiers originating in the *tu-h* stock and interbottle environmental variation.

Females expressing the trait were also taken from the backcross cultures (columns e, f, and g) and crossed with inbred *tu-h* males. The results (column i)

showed that high proportions of abnormal flies, similar to those of the inbred *tu-h* stock, were obtained from most cultures. In one case, however, the female came from a culture (column e, Table 1) in which two percent was abnormal. Six percent of her progeny (column i) was found to be abnormal. Two abnormal females were taken from the culture with six percent and backcrossed again with *tu-h* males from the inbred stock. Each culture (not shown in tables) had less than two percent of abnormal flies. Presumably *tu-1* was heterozygous in the female producing the six percent culture and also in the two females producing less than two percent. The six percent was attributed to the independent action of *tu-3* in homozygous condition, modifiers, and a bottle environment favorable to the expression of the trait.

The next backcrosses were made between abnormal females (from cultures listed in column g) and Oregon males. In the results of the first backcross (column j) with Oregon as the recurrent parent most cultures had about 30 percent or more of abnormal flies. These were from mothers presumably homozygous for *tu-1* and carrying *tu-3* (heterozygous) and modifiers. A wide variation from nine to 80 percent was obtained from this backcross. It was attributed to interbottle environmental variation and segregation of *tu-3* genes and modifiers. From the second backcross of this series (column k), for which abnormal females were taken from the cultures of the first backcross (column j) and males from the inbred Oregon stock, no abnormal flies were obtained. This is because all mothers were heterozygous for *tu-1*.

A final check was made by crossing Oregon females with abnormal males from the seventh backcross to *tu-h* (from cultures listed in column g). This cross should be similar to the original cross between Oregon females and *tu-h* males. Similar results (column 1) were obtained. No abnormal flies were observed.

The results of the second experiment are summarized in Tables 3 and 4. In general the results are comparable with those of the first experiment but higher percentages of abnormal flies were obtained. In the first backcross cultures, i.e., $F_1 \times$ *tu-h* (column a), considerably more variation was observed and the percentages of abnormal flies were higher than those from the similar cross in the first experiment. Abnormal females and normal appearing females gave similar results (Cf. column a and the column preceding a in Table 3). The difference between the first and second experiment was attributed to modifiers. Crosses were made to determine whether the modifiers were introduced by the special *tu-h* stock used in this experiment or the Canton-S stock. Five F_1 females from *tu-h* $\times$ Canton cultures and 11 from Canton $\times$ *tu-h* were backcrossed to Canton males. No abnormal flies were produced in the 16 cultures. This indicated that the modifiers were recessive and that they were introduced through the special *tu-h* line used in this experiment and not from the Canton stock. Presumably these are the modifiers responsible for the increase from 79 to 93 percent in the *tu-h* line. The increased expression in the results of the second backcross (column b) may also be attributed to these modifiers.

Evidence for segregation of *tu-1* was observed in the results of the second back-

TABLE 3

Crosses between tu-h *females and Canton males and two successive backcrosses to* tu-h *males followed by two successive backcrosses to Canton males**

Inbred *tu-h*	F_1	*tu-h* F_1 × *tu-h*	+ F_1 × *tu-h* a	+ from a × *tu-h* b	*tu-h* from b × Canton c	*tu-h* from c × Canton d
		Percent abnormal progeny from pair matings				
93	44	3	2	0	42	0
	53	6	5	19	56	0
	32	1	1	44	36	0
	38	1	9	0	27	0
	21	3	1	94	41	0
	28	2	0	25	69	0
	35	1	5	0	81	0
	29	1	7	2	25	0
	38	0	7	84	23	0
	52	6	21	0	24	0
		Ratio (from entire experiment): Heterozygous to homozygous mothers 8:15				

* To save space only the first ten percentages are given in each column.

TABLE 4

Crosses between Canton females and tu-h *males and two successive backcrosses to* tu-h *males followed by two successive backcrosses to Canton males**

Inbred *tu-h*	F_1	+ F_1 × *tu-h* a	+ from a × *tu-h* b	*tu-h* from b × Canton c	*tu-h* from c × Canton d
		Percent abnormal progeny from pair matings			
93	0	9	0	12	0
	0	1	0	45	0
	0	4	26	37	0
	0	1	37	14	0
	0	1	88	34	0
	0	1	72	28	0
	0	3	93	21	0
	0	0	0	30	0
	0	11	18	40	0
	0	20	4	24	0
		Ratio (from entire experiment): Heterozygous to homozygous mothers 10:13			

* To save space only the first ten percentages are given in each column.

cross (column b). From the total of 46 pair matings between heterozygous mothers and fathers carrying *tu-1* at least 18 behaved as expected for heterozygous females and 28 showed results which by comparison with the first experiment would be classed with those of homozygous mothers. The modifiers present in this cross make it more difficult to distinguish the cultures from heterozygous

and homozygous mothers. The culture with four percent (column b Table 4) has been classed with those from heterozygous mothers. The cultures with 18, 19, and perhaps 25 and 26 percent could be in this category. When the experimental result of 18:28 was compared with the 1:1 expectation a chi-square value of 2.18 (P= .10–.20) was obtained. There is no significant difference between the results of this experiment and the 1:1 ratio expected on the basis of the hypothesis being tested.

DISCUSSION AND CONCLUSIONS

In general the results of the two experiments conformed to expectations based on the maternal effect hypothesis. No persistent cytoplasmic factor could be detected. Such a factor would be expected to continue its influence through the female line following the cross between *tu-h* females and wild males. The same effect would not be expected in the results of backcrosses following the reciprocal cross between wild females and *tu-h* males. The comparable results from the backcrosses following the reciprocal crosses indicated that no permanent property was transmitted in the eggs. The backcrosses following both reciprocal crosses showed evidence of segregation of a recessive gene (*tu-1*) which was required in the mothers for the increased expression of the trait. The segregation of this gene will explain the difference obtained from reciprocal crosses. The term maternal effect, as defined above, seems appropriate for the case of tumorous head.

It was predicted on the basis of data already available that outcrosses involving mothers homozygous for *tu-1* and *tu-3* and fathers not carrying either of the *tu-h* genes would produce about 30 percent of abnormal flies. From 13 such matings (F_1 Table 1) an average of 32 percent was obtained. Only very small percentages were expected from the reciprocal cross involving wild type females. No abnormal flies were observed in the 20 cultures examined (F_1 Table 2). When the mothers were homozygous for *tu-1* and *tu-3* and a *tu-3* gene was introduced by the male or modifiers were introduced by either parent, percentages higher than about 30 percent were expected. Numerous examples in which such combinations could be present were observed in the results of the backcrosses.

When *tu-1* was homozygous in the mother and *tu-3* was homozygous in both parents, results similar to those from the inbred *tu-h* stock were expected. Numerous examples were observed in the backcrosses in which these gene combinations could have been present and about 79 percent of abnormal flies were produced. When *tu-1* was homozygous in the mother and *tu-3* was segregating in such a way that about one half of the progeny might have two and one half have one *tu-3*, about 50 percent of abnormal flies was expected. When the mothers were similar but *tu-3* was segregating in such a way that one half of the progeny would receive one and one half have no *tu-3* genes about 15 percent of the progeny was expected to express the trait. Examples of these percentages in cultures which could have the various combinations of the major genes and modifiers were observed in the backcross results.

When the mothers were not homozygous for *tu-1* a low penetrance, up to about

three percent, was attributed to the independent action of *tu-3*. When this gene was heterozygous less than one percent of abnormal flies was expected. In homozygous condition this gene could produce one to three percent of abnormal flies. Modifiers of *tu-3* are known to increase the expression. The low percentages of expression observed in the results of the backcrosses were explained on the basis of the independent action of *tu-3* modifiers. When no *tu-3* genes were present, no abnormal flies were expected. These expectations were obtained when *tu-3* was removed.

The *tu-1* gene in the mother is responsible for producing more than a small percentage of abnormal flies. This gene is completely recessive. It must act by conditioning the eggs in some way while they are developing in the mother. A substance may be produced under the influence of the *tu-1* gene which predetermines the developing eggs in such a way that they are more susceptible to the action of the *tu-3* gene. No substance has been detected at the present time. Experiments designed to identify steps between the gene and the character are in progress. Both males and females express the tumorous head trait. The penetrance and expressivity, however, are slightly higher in the females than in the males (GARDNER and RATTY 1952).

Several cases of maternal effect are recorded in the literature. One of the earliest to be investigated was that associated with the direction of shell coiling in snails (*Limnaea peregra*) discovered from the breeding experiments of BOYCOTT, DIVER, and GARSTANG and interpreted by STURTEVANT (1923). In this case the direction of coiling is determined by the orientation of the spindle in the second cleavage of the egg which is predetermined by a gene in the mother.

Another maternal effect is associated with pigmentation in the moth *Ephestia kühniella* (KÜHN, CASPARI and PLAGGE 1935). This example has some features in common with tumorous head in *Drosophila melanogaster* but it seems to be less complex. A dominant gene (*A*) controls the pigmentation of the skin, eyes, and other structures of the larvae and adult moths. When dark-eyed females heterozygous (*Aa*) for the gene are mated with red-eyed males (*aa*) all of the progeny have dark pigment while they are in the larval stage. This is because the substance kynurenine which is associated with the gene (*A*) in the mother influences all larvae. As the larvae develop, the maternal effect disappears and the pigmentation in the adults is determined by the genes of the individual moths. One half of the adults from the cross $Aa \times aa$ have dark eyes and one half have red eyes. When ovaries from *aa* moths are implanted into *AA* or *Aa* females and the females are mated with *aa* males the *aa* offspring have dark eyes at first but they gradually become lighter as the moths mature. This suggests that the eggs are influenced early by the *AA* maternal surroundings. Kynurenine is apparently taken up by the eggs from the surroundings in the mother. The effect is only temporary. The original phenotype influenced by the mother is replaced by the one controlled by the genes of the individual as development proceeds.

Other maternal effects have been discovered in the silk worm *Bombyx mori* (TANAKA 1924) and fruit fly *Drosophila melanogaster* (GOLDSCHMIDT 1951;

GOLDSCHMIDT, HANNAH and PITERNICK 1951) *D. pseudoobscura* (DOBZHANSKY 1935) and Drosophila interspecific hybrids (DOBZHANSKY and BOCHE 1933; STURTEVANT 1946).

SUMMARY

Two experiments involving series of backcrosses were conducted to determine whether a maternal effect or a cytoplasmic factor is responsible for the difference in reciprocal crosses observed for the tumorous head trait in *Drosophila melanogaster* and whether the genetic mechanism can be explained by the segregation of a single recessive gene. The results supported the maternal effect hypothesis. A sex-linked, recessive gene controls the maternal effect. An autosomal semi-dominant gene is necessary for any expression of the trait.

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THE DIRECT ESTIMATION OF MUTATION RATE FROM MUTANT FREQUENCY UNDER SPECIAL CONDITIONS

MAURICE OGUR, RALPH ST. JOHN, SYLVIA OGUR AND ABRAHAM M. MARK

Biological Research Laboratory and Department of Mathematics, Southern Illinois University, Carbondale, Illinois

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THEORETICAL analyses of the growth of microbial populations containing two types of individuals, subject to mutation from one to the other based upon either deterministic or probabilistic models, have been made by LURIA and DELBRUCK (1943); SHAPIRO (1946); ARMITAGE (1956) and others. Where the growth and mutation rates of normal and mutant cells are unequal, a complex situation arises with four independent growth and mutation rate constants. Where selection or backmutation are excluded, the situation is simplified.

In the current study we introduce an extreme simplification; total selection against the mutant. Exclusion of mutant growth and backmutation can be shown to produce a population equilibrium in which the mutant frequency is numerically equal to the mutation rate.

If $n=n(t)$ is the number of N (normal) cells at time t, and $m=m(t)$ is the number of mutant cells at time t, then we write

$$(1)\quad \frac{dn}{dt}=kn$$

and

$$(2)\quad \frac{dm}{dt}=an$$

Equation (1) expresses the rate at which N cells produce N offspring with k defined as the rate constant in this process. The overall growth rate would be represented by $k+a$. Equation (2) asserts that the rate at which the M population is increasing depends only on the number of N cells available for mutation with a defined as the mutation rate constant. Since M cells cannot divide in the selective growth medium they arise only by mutation of N cells.

These equations are based essentially upon the conventional deterministic formulation of the laws of population increase. Alternatively, in a stochastic formulation they may be shown to represent the mean numbers of N and M cells at time t.

From (1) we have

$$(3)\quad n=n_o e^{kt}$$

where n_o is the number of N cells at time $t=o$. Inserting (3) into (2) we have

$$\frac{dm}{dt}=a\,n_o\,e^{kt}$$

whence

$$(4)\quad m=\frac{a\,n_o\,e^{kt}}{k}+\left(m_o-\frac{a\,n_o}{k}\right)$$

where m_o is the number of M cells at time $t=o$. Combining (3) and (4) we have

$$\frac{m}{n+m}=\frac{\frac{a}{k}\,n_o+\left(m_o-\frac{a\,n_o}{k}\right)e^{-kt}}{\left(1+\frac{a}{k}\right)n_o+\left(m_o-\frac{a\,n_o}{k}\right)e^{-kt}}$$

whence

$$(5)\quad \lim_{t\to\infty}\ \frac{m}{n+m}=\frac{a}{k+a}$$

Thus, when population equilibrium has been reached after growth in exponential phase in a medium totally selective against the mutant, the relative mutant frequency equals the mutation rate. Expressions comparable to equation (5) may also be obtained by imposing the limiting conditions of no mutant growth or back-mutation upon derivations of SHAPIRO (1946); ARMITAGE (1952) or MARCOVICH (1953).

The spontaneous cytoplasmic mutation to respiration deficiency (*aer*) in yeast (EPHRUSSI 1953) was chosen to test the analysis because it represented an experimental system in which the imposed limitations could be very nearly realized. The *aer* mutants apparently arise only during growth and do not revert to respiration sufficient (*AER*) cells under all conditions which have been tested. Media totally selective against the *aer* mutant have been constructed with carbon sources which are oxidized but not fermented (OGUR, LINDEGREN and LINDEGREN 1954; OGUR and ST. JOHN 1956).

The relative error in estimating mutant frequency arising from the budding of yeasts

Ideally, estimates of mutant frequency by plating methods should be based upon colonies which have arisen from single cells. To estimate the error introduced by plating yeasts with attached buds, the budding patterns of a number of strains during the exponential phase of growth in the selective medium at 30°C were studied. Two principal patterns were observed. With a tetraploid strain, (case I) the configurations consisted almost exclusively of either doublets or singlets (groups of attached cells will be referred to as configurations). It may be inferred that this strain grows under these conditions by budding of single cells with the resultant doublets splitting to singlets before the beginning of a second budding cycle (a complete generation extends from singlet to singlet). A number of diploid strains, however, exhibited very few singlets, a preponderance of doublets and some triplets and quadruplets, with only infrequent occurrence of any larger configurations. (See also EPHRUSSI, HOTTINGUER and CHIMENES 1949.) The pattern (case II) seems to involve the budding of a doublet to form a triplet, and then in most cases a quadruplet, which splits to form two doublets (a complete generation extends from doublet to doublet). This inference was con-

firmed qualitatively by direct observation of budding in hanging drop preparations. The relative frequency of doublets, triplets, and quadruplets may be assumed to reflect their relative average time duration in the budding cycle. The infrequent occurrence of configurations larger than quadruplet indicates that any more complex growth patterns should have no significant effect on population analysis unless it is preferential for configurations containing mutant buds. We have no evidence bearing on the latter assumption.

The two budding patterns described above, and their effects on the production and expression of *aer* mutants, are represented diagrammatically in Figure 1 for the case of growth in the selective medium in which mutants form but do not divide.

It will be noted that in case I the mutant bud formed in a given budding cycle will be expressed at the end of that cycle when the mutant separates from its mother cell. In nonsynchronous populations the estimate of mutant frequency will be biased, however, if mutants in mixed doublets are not expressed. (This possibility will be considered again in relation to the appearance of variegated colonies.)

In the case II pattern mutant buds may arise only at the triplet or quadruplet stage and do not express themselves at the end of the generation in which they are produced, but after a bud separation lag of one additional generation. This is analogous to segregational lag in the expression of mutation of nuclear origin in binucleate microorganisms (LEDERBERG 1949).

We have attempted to evaluate the errors in estimation of mutant frequency for both budding patterns.

Let $n_1, n_2, n_3, n_4, m_1, m_2, m_3, m_4$, stand for the number of normal (N) and mutant (M) configurations shown in Figure 1.

Let T denote the total number of configurations.

Let f denote the relative mutant frequency based on configurations.

Let F denote the relative mutant frequency based on cells rather than configurations.

We are interested in the relative error

$$E = \frac{f - F}{F} = \frac{f}{F} - 1$$

Case I

$$T = n_1 + n_2 + m_1 + m_2 \qquad f = \frac{m_1}{T}$$

$$F = \frac{m_1 + m_2}{n_1 + 2n_2 + m_1 + 2m_2} = \frac{m_1 + m_2}{T + n_2 + m_2}$$

Let C_2 = the relative frequency of doublets $= \dfrac{n_2 + m_2}{T}$

Then $T = \dfrac{n_2 + m_2}{C_2}$

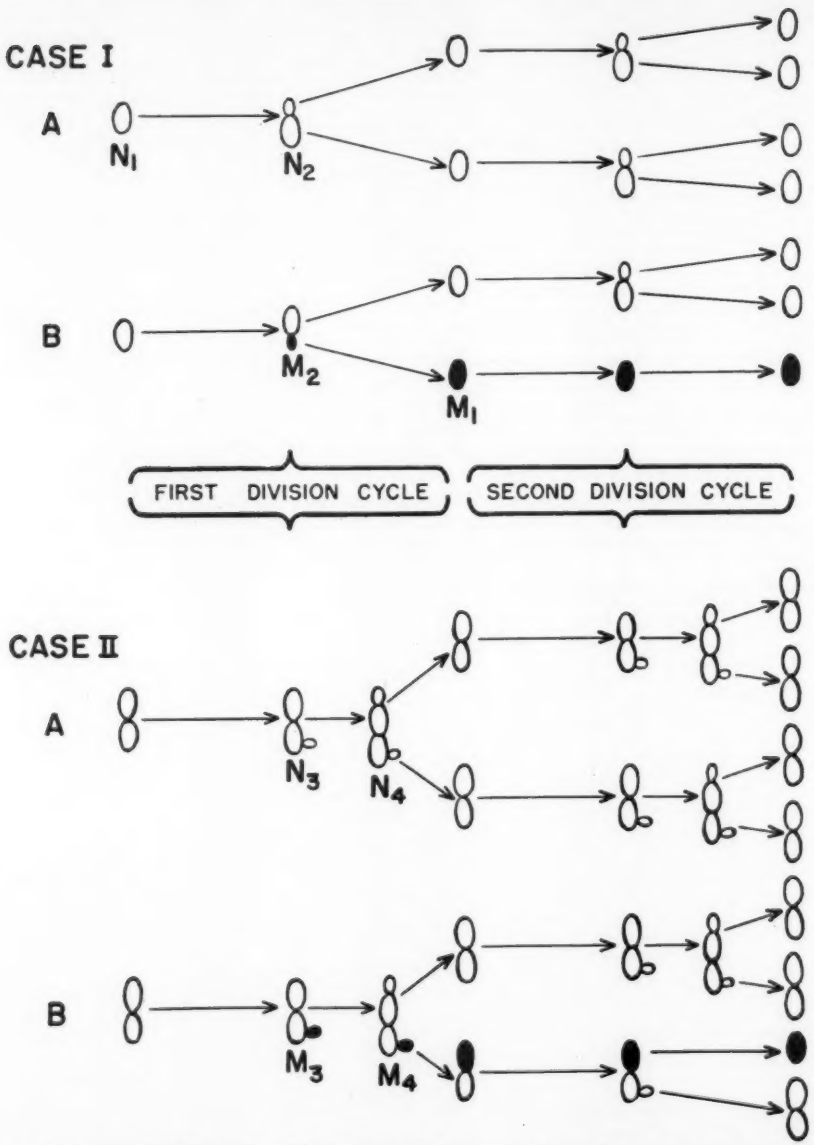

FIGURE 1.—Budding patterns of yeast strains in exponential growth in a selective medium. Shaded cells are mutant; unshaded cells are normal.

N_1 = normal singlet; N_2 = normal doublet; N_3 = normal triplet; N_4 = normal quadruplet. M_1 = mutant singlet; M_2 = mixed doublet; M_3 = mixed triplet; M_4 = mixed quadruplet.

and

$$f=\frac{m_1C_2}{n_2+m_2} \qquad F=\frac{(m_1+m_2)C_2}{(n_2+m_2)(C_2+1)}$$

$$\frac{f}{F}=\frac{(m_1C_2)(n_2+m_2)(C_2+1)}{(n_2+m_2)(m_1+m_2)(C_2)}=(C_2+1)\left(\frac{m_1}{m_1+m_2}\right)$$

$$Let\ \alpha=\frac{\text{the number of mixed doublets}}{\text{the total number of mutants}}=\frac{m_2}{m_1+m_2}$$

Then

$$E=\frac{f}{F}-1=(C_2+1)(1-\alpha)-1$$

We note that α has a maximum possible value of 0.5 when all last generation mutants are present as doublets. As $C_2 \to 1$ and $\alpha \to 0.5$. $E \to 0$. Thus in a population consisting overwhelmingly of doublets, with mutant doublets unexpressed, the errors in estimation of mutant and total populations are compensatory.

TABLE 1

Calculated errors in estimating mutant frequency due to budded cells (case I)

C_2*	1	0.83	0.80	0.75	0.66	0.5	0.33	0.1	0
α†	0.5	0.42	0.40	0.38	0.33	0.25	0.17	0.05	0
E‡	0	7%	8%	9.4%	11%	12.5%	11%	4.5%	0

* C_2 = the relative frequency of doublets $=\frac{n_2+m_2}{T}$

† $\alpha=\frac{\text{the number of mutant doublets}}{\text{the total number of mutants}}=\frac{m_2}{m_1+m_2}$

‡ E = the relative error $=(C_2+1)(1-\alpha)-1$

Table 1 shows the calculated relative errors arising from various values of C_2 between 0 and 1. Values of α have been paired with values of C_2 on the assumption that the decrease in the proportion of mutant doublets will parallel the decrease in the proportion of all doublets. C_2 can be evaluated by direct counts at the time of plating. There appears to be no convenient way to evaluate α experimentally, but it is apparent that in nonsynchronous division it would be somewhat smaller than 0.5 and would tend to decrease with C_2. If α does not decrease as rapidly as C_2, the calculated error will be somewhat smaller. In any case. it seems conservative to conclude that the maximum probable relative error in the estimation of mutant frequency due to budding in the case I pattern will be less than 20 percent.

Case II

$$T=n_1+n_2+n_3+n_4+m_1+m_2+m_3+m_4$$

$$f=\frac{m_1}{T} \qquad F=\frac{m_1+m_2+m_3+m_4}{(n_1+m_1)+2(n_2+m_2)+3(n_3+m_3)+4(n_4+m_4)}$$

Let C_1, C_2, C_3, C_4 be the relative frequency of singlets, doublets, triplets and quad-

ruplets so that

$$C_1=\frac{n_1+m_1}{T} \quad C_2=\frac{n_2+m_2}{T} \quad C_3=\frac{n_3+m_3}{T} \quad C_4=\frac{n_4+m_4}{T} \quad C_1+C_2+C_3+C_4=1$$

Let C_5 = the relative frequency of unexpressed mutants occurring in mixed configurations $\frac{m_2+m_3+m_4}{T}$

Then

$$F=\frac{m_1C_5T}{C_1T+2C_2T+3C_3T+4C_4T}$$

and

$$F=\frac{f+C_5}{C_1+2C_2+3C_3+4C_4}=\frac{f+C_5}{1+C_2+2C_3+3C_4}$$

The quantity $C_2+2C_3+3C_4$ can be evaluated experimentally by direct count of the relative frequency of doublets, triplets and quadruplets. In a series of experiments with diploid yeasts, growing in exponential phase according to the case II pattern, this quantity appeared to be relatively constant (1.20–1.60).

The evaluation of C_5 is difficult, but a maximum of $C_5=3f$ would express the limit if all mutants of the last two generations before plating were present in mixed configurations and were unexpressed.

Taking a mean value of 1.40 for $C_2+2C_3+3C_4$ we get

$$F=\frac{f+3f}{2.40}$$

$$E=\frac{f}{F}-1=\frac{2.40}{4}-1=-40 \text{ percent}$$

Thus, with the more complex budding pattern of case II, the maximum probable relative error due to the presence of configurations larger than singlets is −40 percent.

The relative error arising from phenomic lag

If newborn mutants are capable of several divisions in the selective medium before their respiratory capacity is diluted out, an upward bias will be imposed on the mutant frequency. Assuming that the growth rate of mutants in phenomic lag depends directly on residual respiratory capacity which is partitioned equally in four or more phenomic lag generations the bias can be calculated to be + 78 percent. Since some of the mutants in phenomic lag must be present as doublets, a more correct estimate would be somewhat smaller (ca. + 50 percent). The errors introduced by the case II budding pattern (ca. −40 percent) and phenomic lag are thus partly compensating. Phenomic lag which occurs after plating does not influence the estimate of mutant frequency since colony type is diagnosed after four days, at which time all mutant configurations have become mutant clones.

We, therefore conclude that the estimation of mutant frequency for yeasts following the aforementioned budding patterns is not unreasonable. The more complex budding habit of most haploid yeasts in exponential growth makes pre-

cise population estimates of mutant frequency in haploid strains somewhat dubious at present.

MATERIALS AND METHODS

The yeasts employed were diploid cultures from the Carbondale collection of inbred Saccharomyces strains. They were chosen for reasonably close adherence to the case II budding pattern.

The storage medium was yeast extract-peptone nutrient agar containing one percent glucose. The selective medium differed only by the substitution of sodium lactate (approximately one percent end conc., pH 5.5) for glucose, and by omission of agar (See OGUR and ST. JOHN 1956 for details of media composition).

Inocula from storage slants were grown 24 hours on glucose nutrient agar. Cells were harvested and transferred to 250 ml Erlenmeyer flasks containing 40 ml of the selective medium at an initial concentration of 10^3 cells per ml. Flasks were incubated in a Dubnoff shaking incubator at 30°C. Samples taken at intervals were spread on glucose nutrient agar after appropriate dilution, to yield between 100 and 200 colonies per plate. Plates were incubated at 30°C for four days, then overlaid with agar containing 2.3.5 triphenyltetrazolium chloride (TTC) (OGUR, ST. JOHN and NAGAI 1957). Red (*AER*) and white (*aer*) colonies were scored three hours after overlay and the mutant frequency estimated as the ratio of white colonies to the total number of colonies.

In addition to red and white colonies some sectored or variegated colonies were observed. These are interpreted as having arisen from mixed configurations present at plating, or during the first few divisions on the plate. The principles of our derivation require that variegated colonies arising from an event preceding plating be scored as mutant, whereas those arising from a division on the plate be scored as normal. Distinction between these alternatives is difficult. If every mutant bud arising either in the last few generations before plating or in the early divisions on the plate produced a variegated colony. it would follow that the number of variegated colonies should exceed the number of white colonies. This was not the case. It must be inferred that variable patterns of colonial growth may obscure the variegation in some cases, or stated conversely, sectoring depends not only on the relatively early development of heterogeneity but also on an orderly pattern of colonial growth for its expression. (Some strains with relatively high mutation rate have been found to produce heterogeneous colonies which nevertheless exhibited grossly homogeneous but lighter formazan color after TTC overlay.)

In practice, the small number of variegated colonies was scored as normal since there is a somewhat greater probability that they were formed after rather than before plating.

Population equilibrium in the selective medium

To study the conditions for achievement of population equilibrium, a yeast strain (14940) which exhibited a high spontaneous mutation rate (ca. 2×10^{-5} per cell per generation) was studied in a series of experiments. Cells, grown

under a variety of initial conditions, were inoculated into the selective medium. Figure 2 summarizes the results of a 96-hour experiment in which the inoculum contained approximately 55 percent of *aer* cells. Exponential growth was maintained by transfer of ca. 10^4 cells to fresh medium every 24 hours. Results have been calculated back to unit volume in the original flask. The uppermost curve represents the growth of the entire population based upon the total colony count. The other two curves are based upon the red and white colony counts respectively. Growth in the selective medium produced a decrease in the mutant frequency to the equilibrium value (ca. two percent) by 32 hours. This frequency was maintained in subsequent growth in the selective medium and the curves based upon total, red and white colonies are parallel between 32 and 96 hours.

Figure 3 summarizes a similar experiment in which cells of the same strain, after three 24-hour transfers in the selective medium, were harvested, washed sterilely and reinoculated into the selective medium. After some initial lag, exponential growth and an equilibrium population were again established and maintained for the duration of the experiment to 96 hours.

These results are consistent with the theoretical derivation. One can calculate, for given values of initial and equilibrium populations, the number of generations required to reach a mutant frequency within five percent of the equilibrium value. Such calculations yield values between seven and nine generations for the population changes anticipated in the current study, or within 24 hours under the specified growth conditions. In subsequent routine estimations of the mutation rate, exponential growth through at least 72 hours in the selective medium was employed.

It seemed of interest to test the effect of carrying an equilibrium population into stationary phase on both the observed mutant frequency and the frequency of various budding configurations. In several experiments, flasks, from which transfers had been made at 24 hours, were not discarded but were reincubated and analyzed at 48, 72 and 96 hours. As the culture was carried into the stationary phase, a trend was observed toward simpler configurations. Whereas at 24 hours in exponential phase, strain 14940 in one experiment exhibited only two percent of its cells as singlets and 62 percent as doublets (case II), by 96 hours in the stationary phase, singlets comprised 53 percent and doublets 26 percent of the total population (approaching case I). The mean mutant frequency calculated from several samples from the exponential phase was approximately a third lower than the mean mutant frequency calculated from a number of samples from the stationary phase. (It will be recalled that the calculated maximum probable relative error for the case II budding pattern was —40 percent.

Although our derivation is based upon exponential growth, it seems likely that analysis of the equilibrium population after it had been carried into stationary phase would compensate in part for the error introduced by the case II budding pattern. We have preferred as the safer procedure, however, to estimate mutation rates from data obtained in the exponential phase.

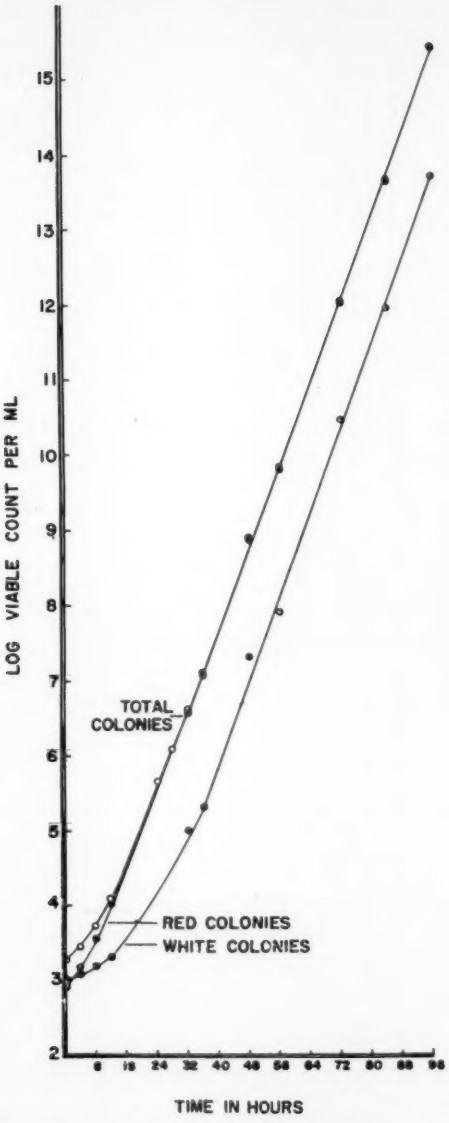

FIGURE 2.—Equilibrium population achieved by strain 14940 during exponential growth in a medium selective against the mutant. The initial population contained a high frequency of *aer* cells.

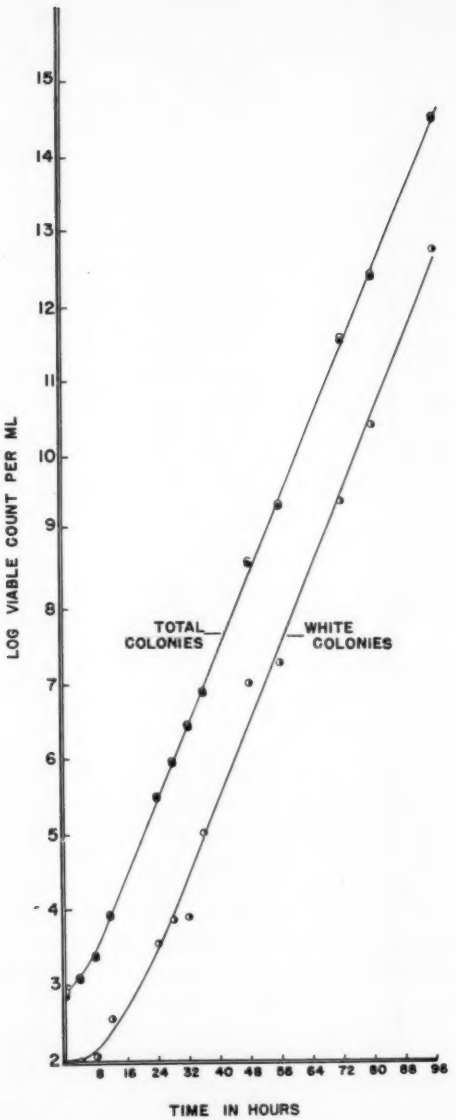

FIGURE 3. Equilibrium population achieved by strain 14940 from an initial population containing a relatively low frequency of *aer* cells.

Mutation rates of various yeast strains from the mutant frequency at population equilibrium

Several strains which adhered most closely to the case II budding pattern were chosen for further study. Some were heterozygous for adenine deficiency (*ad*), and served as controls against the possibility that meiosis and sporulation, or some other segregational mechanism, might have intervened during the prolonged incubation in the selective medium. Microscopic examination revealed no asci and no pink (*ad*) colonies were found among the thousands scored on diagnostic plates.

Routinely, strains were inoculated into the selective medium and carried by daily transfer through 72 hours in exponential growth. Mutant frequencies were estimated as previously described. Each estimate was based on a minimum of 40 plates. To insure the achievement of equilibrium, replicate determinations were performed on the same cultures carried for 72 additional hours in exponential growth.

Table 2 summarizes the results of replicate determinations of the spontaneous mutation rate to respiration deficiency at 30°C. Strain dependent mutation rates from 1×10^{-3} to 16×10^{-3} mutations per cell per generation are observed. Using different strains and methods, values ranging from 2×10^{-3} to 40×10^{-3} have been reported by EPHRUSSI, L'HERITIER and HOTTINGUER (1949); MARCOVICH (1953); and LASKOWSKI (1954). The latter's methods required micromanipulative isolation of single cells, growth to clones of 100–200 cells, spreading on the surface of agar and estimation of the number of plates containing no mutants with colony size as the diagnostic criterion (confirmed in some cases by the Nadi test or spectroscopic examination of individual clones).

TABLE 2

Rates of spontaneous mutation to respiration deficiency of various yeast strains

Yeast strain	Number of colonies		Mutation rate*
	Total	Mutant	
14,716 × 8,256	11,780	14	1.2
	10,970	18	1.6
8,324 × 8,282	12,720	66	5.2
	11,713	35	3.0
15,220 × 8,256	6,647	9	1.4
	7,018	4	0.6
	12,266	13	1.1
14,940	6,934	113	16.
	6,103	80	13.
	10,791	216	20.
13,516	19,887	73	3.7
	24,246	112	4.6

* Mutations per cell per generation × 10^5.

DISCUSSION

Although the mutant frequency in a microbial population does not generally represent the mutation rate, it may under special conditions. These conditions are met, in theory, after prolonged growth under conditions where mutants can neither divide nor backmutate. It must be further assumed that the equilibrium mutant frequency is maintained solely by these limitations. Cytoplasmic *aer* mutants arising in a totally selective medium reach stable equilibria, as predicted, at frequencies that are strain-dependent and resemble the mutation rates that other investigators have obtained by more laborious methods. This strongly suggests that the system under study has met the requirements of our theoretical analysis.

The observed mutant frequencies may, however, be subject to bias from several sources (e.g., phenomic lag, attached buds, and different death rates of normal and mutant cells). The probable errors introduced by the first two appear to be of comparable magnitude and opposite in sign but the differential death rate bias is difficult to estimate by existing methods.

We have determined the death rate of nondividing mutant cells (from clonal isolates) in the selective medium to be ca. four percent per 24 hours (ca. 0.4 percent per generation of normals). The death rate of normal cells growing exponentially seemed in principle even more difficult to estimate. But a new theoretical approach to this problem may be made if one considers that any medium is in fact totally selective against dead cells. This produces a situation comparable to the one already considered in this report for a mutational process; continued exponential growth should produce an equilibrium frequency of dead cells numerically equal to the death rate. The application of this principle will however, require more satisfactory experimental methods for estimation of low frequencies of dead cells by direct count rather than by difference between hemacytometer and plate counts. Our calculations indicate, however, that the bias introduced by anticipated death rates will be small.

A danger inherent in any "long term" population experiment is based upon the possible appearance of a third type of individual, better adapted to the growth conditions, which could replace the original cell type. We have found no evidence that this had occurred with the strains studied.

It is interesting to consider the possibility of a general experimental solution of the problem of estimating mutation rates based upon these principles. Thus, if the culture were grown to population equilibrium in a medium totally selective against the mutant and the mutant frequency were estimated by plating on a medium totally selective for the mutant it might be possible to extend the method to mutations occurring at rates too low to be estimated by direct plating. At present, no system in which this approach may be tested is known to us.

Finally, an unusually simple demonstration of mutagenicity (see EPHRUSSI, L'HERITIER and HOTTINGUER 1949 and EPHRUSSI and HOTTINGUER 1950 for comparison) may be based on the selective medium. Any enhancement of the mutant frequency, in strains grown in the selective medium to population equi-

librium in the presence of potential inducing agents or conditions, enables one to conclude that this enhancement has arisen by increasing the mutation rate rather than by selection for the mutant, since the medium is already totally selective against the mutant (provided selective toxicity to the normal has been excluded). In addition to the qualitative conclusion the enhanced mutation rate is simultaneously estimated.

SUMMARY

The growth of a mixed population of normal and mutant cells in a medium totally selective against the mutant has been analyzed theoretically. The analysis predicts the achievement of population equilibrium with mutant frequency numerically equal to the mutation rate. The analysis has been applied to the spontaneous cytoplasmic mutation to respiration deficiency in yeast. Population equilibrium was reached within 32 hours at 30°C. The strain dependent mutation rates estimated by this method fell within the range reported by more laborious methods. The probable error introduced into these estimates by phenomic lag and by plating yeasts with attached buds has been calculated. Possible application of these principles to other systems has been considered.

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CONTENTS OF PRECEDING ISSUES

JANUARY, 1959

DEMEREC, M., Albert Francis Blakeslee.

BINET, F. E., A. M. CLARK, and H. T. CLIFFORD, Correlation due to linkage in certain wild plants.

GILMOUR, D. G., Segregation of genes determining red cell antigens at high levels of inbreeding in chickens.

JUDD, B. H., Studies on some position pseudoalleles at the white region in *Drosophila melanogaster*.

SPIESS, ELIOT B., Release of genetic variability through recombination. II. *Drosophila persimilis*.

MALOGOLOWKIN, CHANA, D. F. POULSON, and E. Y. WRIGHT, Experimental transfer of maternally inherited abnormal sex-ratio in *Drosophila willistoni*.

DOBZHANSKY, TH., H. LEVENE, B. SPASSKY, and N. SPASSKY, Release of genetic variability through recombination. III. *Drosophila prosaltans*.

LEVENE, HOWARD, Release of genetic variability through recombination. IV. Statistical theory.

STADLER, JOAN, and CHARLES YANOFSKY, Studies on a series of tryptophan-independent strains derived from a tryptophan-requiring mutant of *Escherichia coli*.

SIRE, MARJORIE W., and R. A. NILAN, The relation of oxygen posttreatment and heterochromatin to X-ray-induced chromosome aberration frequencies in *Crepis capillaris*.

MORPURGO, GIORGIO, and GIUSEPPE SERMONTI, Chemically-induced instabilities in heterozgous diploids of *Penicillium chrysogenum*.

MARCH, 1959

GLASSMAN, EDWARD, and H. K. MITCHELL, Mutants of *Drosophila melanogaster* deficient in xanthine dehydrogenase.

GERSH, EILEEN SUTTON, Some observations on chromosome pairing in the salivary gland nuclei of *Drosophila melanogaster*.

ABRAHAMSON, SEYMOUR, The influence of oxygen on the X-ray induction of structural changes in Drosophila oocytes.

LINDSLEY, D. L., and E. NOVITSKI, Compound chromosomes involving the X and Y chromosomes of *Drosophila melanogaster*.

HANSON, W. D., The theoretical distribution of lengths of parental gene blocks in the gametes of an F_1 individual.

MUKAI, TERUMI, and ALLAN B. BURDICK, Single gene heterosis associated with a second chromosome recessive lethal in *Drosophila melanogaster*.

SANDLER, L., YUICHIRO HIRAIZUMI, and IRIS SANDLER, Meiotic drive in natural populations of *Drosophila melanogaster*. I. The cytogenetic basis of segregation-distortion.

JOHN, B., and K. R. LEWIS, Selection for interchange heterozygosity in an inbred culture of *Blaberus discoidalis* (Serville).

DEMEREC, M., and H. OZEKI, Tests for allelism among auxotrophs of *Salmonella typhimurium*.

FABERGÉ, A. C., Production by alpha particles of functionally stable broken chromosome ends in maize.

MAY, 1959, PART 1

TANTAWY, A. O., Selection limits with sib-matings in *Drosophila melanogaster*.

JINKS, J. L., and J. M. STEVENS, The components of variation among family means in diallel crosses.

CAMPBELL, ALLAN, and EVELYN BALBINDER, Transduction of the galactose region of *Escherichia coli* K12 by the phages λ and λ-434 hybrid.

HERSKOWITZ, I. H., H. J. MULLER, and JOHN S. LAUGHLIN, The mutability of 18 Mev electrons applied to Drosophila spermatozoa.

HERSKOWITZ, IRWIN H., Induced changes in female germ cells of Drosophila. VI. The effect of dehydration upon the X-ray induced frequencies of crossover-like exchanges and of gross chromosomal rearrangements.

SPRAGUE, G. F., W. A. RUSSELL, and L. H. PENNY, Further studies on convergent improvement in corn.

CARLSON, ELOF AXEL, Allelism, complementation, and pseudoallelism at the dumpy locus in *Drosophila melanogaster*.

TAKAHASHI, TOSHIAKI, and YONOSUKE IKEDA, Bisexual mating reaction in *Saccharomyces chevalieri*.

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